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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/00, 38/02, 38/17, 39/395

A1

(11) International Publication Number: **WO 98/01145**

(43) International Publication Date: 15 January 1998 (15.01.98)

(21) International Application Number: PCT/US97/12925

(22) International Filing Date: 3 July 1997 (03.07.97)

(30) Priority Data:
08/677,730 8 July 1996 (08.07.96) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/677,730 (CIP)
Filed on 8 July 1996 (08.07.96)

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THERAPEUTIC APPLICATIONS OF T-BAM (CD40L) TECHNOLOGY TO TREAT DISEASES INVOLVING SMOOTH MUSCLE CELLS

(57) Abstract

Activation by CD40 ligand (CD40L) of smooth muscle cells bearing CD40 on the surface of the cells is inhibited in vivo and ex vivo by contacting the cells with an agent capable of inhibiting interaction between CD40L and CD40 on the cells. In vivo inhibition of CD40-bearing smooth muscle cells is used to treat smooth muscle cell-dependent diseases.

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THERAPEUTIC APPLICATIONS OF T-BAM (CD40L) TECHNOLOGY TO TREAT DISEASES INVOLVING SMOOTH MUSCLE CELLS

This application claims the priority of United States Patent Application Serial No. 08/677,730, filed July 8, 1996 the contents of which is hereby incorporated by reference into the present application.

The invention disclosed herein was made with Government support under NIH Grant Nos. K08-AR-01904, RO1-CA55713, RO1-AI-28367, RO1-AI-14969, HL21006, HL42833, HL50629, and RO1-AI-14969 from the Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found in the text or listed by number following the Experimental Details section.

Background of the Invention

CD40 is a cell surface molecule expressed on a variety of cells and interacts with a 30-33 kDa activation-induced CD4+ T cell counterreceptor termed CD40L. CD40L-CD40 interactions have been extensively studied in T cell-B cell interactions and are essential for T cell dependent B cell differentiation and IgG, IgA and IgE production. CD40 is also expressed on monocytes, dendritic cells, epithelial cells, endothelial cells and fibroblasts. CD40 expression on these cells is upregulated in vitro by cytokines, most notably IFN-γ. Interestingly, in vivo studies have demonstrated markedly upregulated CD40 expression in inflammatory sites, such as rheumatoid

arthritis synovial membrane or psoriatic plaques. In vitro studies utilizing anti-CD40 mAb or CD40L+ cells demonstrate that CD40 is functionally expressed on monocytes, dendritic cells, epithelial cells, endothelial cells and fibroblasts.

For example, CD40L-CD40 interactions induce monocytes to secrete the proinflammatory cytokines IL-Iα, IL1ß, IL-6 and TNF-α and dendritic cells to secrete TNF-α. CD40L-CD40 interactions also promote monocytes and dendritic cells to secrete the chemokines IL-8 and MIP1α. Moreover, CD40 ligation enhances IL-1 mediated GM-CSF production by thymic epithelial cells. Additionally, CD40L mediated signals induce monocytes to secrete IL-10 and nitric oxide and augment fibroblast IL-6 production. Fibroblasts also proliferate following CD40L-CD40 interactions. Finally, endothelial cells and fibroblasts upregulate intercellular adhesion molecules following CD40 ligation.

Vascular diseases such as atherosclerosis have been treated with a variety of drugs, including cholesterol-lowering drugs, beta blockers, calcium channel blockers, and anti-coagulants. It is now demonstrated that smooth muscle cells are competent to express CD40. This provides a basis for treatment of vascular diseases by inhibition of interactions between CD40 and CD40 ligand (also known as T-BAM, 5c8 Ag, gp39, and TRAP). Other diseases involving smooth muscle are also treated by inhibiting CD40-CD40L interactions.

Summary of the Invention

This invention provides a method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells, comprising contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells.

This invention provides a method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells in the subject.

This invention provides a method of treating, in a subject, a smooth muscle cell-dependent disease, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells in the subject and thereby treat the smooth muscle cell-dependent disease.

Description of the Figures

Figure 1A: FACS analysis of resting human aortic smooth muscle cells. The dotted line represents isotype control mAb; the dashed line represents anti-CD54 mAb; and the solid line represents anti-CD40 mAb. This figure shows that smooth muscle cells do not constitutively express CD40.

Figure 1B: FACS analysis of human aortic smooth muscle cells in the presence of IFN-γ (1000 U/cc) after 72 hours in cell culture. This figure shows upregulation of smooth muscle cell CD40 expression in response to IFN-γ.

Figure 1C: FACS analysis of human aortic smooth muscle cells in the presence of IL-1α (1 ng/cc) after 72 hours in cell culture. No upregulation of smooth muscle cell CD40 expression was observed.

Figure 1D: FACS analysis of human aortic smooth muscle cells in the presence of or TNF-α (200 U/cc) after 72 hours in cell culture. No upregulation of smooth muscle cell CD40 expression was observed.

Figures 2A-Y: Atomic coordinates of crystal structure of soluble extracellular fragment of human CD40L containing residues Gly116-Leu261 (in Brookhaven Protein Data Bank format). (SEQ ID NO:1).

Figures 3A-3B: CD40 is expressed in situ on smooth muscle cells and macrophages in lesions of transplant atherosclerosis. Shown are photomicrographs of two-color immunohistochemistry studies demonstrating CD40 expression (brown staining) on smooth muscle cells (red staining) in Figure 3A and macrophages (red staining) in Figure 3B in a patient with transplant related atherosclerosis.

Figures 4A-4B: Normal coronary artery from a patient with idiopathic cardiomyopathy stained with hematoxylin and eosin (Fig. 4A) and anti-CD40 mAb (Fig. 4B). Fig. 4A: Note the absence of intimal thickening or inflammatory infiltrate. Fig 4B: CD40 expression is restricted to endothelial cells lining the vascular lumen. There was no reactivity with an isotype specific control mAb (not shown). (Fig 4A, Fig 4B x25)

Figures 5A-5B: Fibroatheromatous plaque in a coronary artery of a patient with ischemic cardiomyopathy stained with hematoxylin and eosin (Fig 5A) and anti-CD40 mAb (Fig 5B). Fig. 5A: The fibrous cap overlying the partially calcified atheromatous core contains numerous inflammatory cells (arrows). Fig 5B: Most of the inflammatory cells in the fibrous cap are strongly CD40+ (arrows). Adjacent intimal smooth muscle cells and endothelial cells are also CD40+. (Fig 5A, Fig 5B x25)

Figures 6A-6C: Early intimal lesion rich in foam cells in a patient with transplant associated coronary artery disease (TCAD) stained with hematoxylin and eosin (Fig 6A) and anti-CD40 mAb (Fig 6B, Fig 6C). Fig 6A: The intimal lesion contains numerous foam cells, macrophages and smooth muscle cells. Fig 6B: CD40 is strongly expressed on many intimal cells in this early lesion of TCAD. Fig 6C: In particular, foam cells showed abundant staining for CD40. (Fig 6A x25, Fig 6B x50, Fig 6C x400).

Figures 7A-7D: Inflammatory infiltrate present in the fibrous cap of intimal lesion in native CA labelled with anti-CD40L mAb (Fig 7A), control mAb (Fig 7B), anti-CD4 mAb (Fig 7C) and anti-CD8 mAb (Fig 7D). Fig 7A: Characteristic cytoplasmic and cell surface CD40L immunoreactivity which was restricted to lymphocytes. Fig 7B: The same lesion stained with an irrelevant isotype

matched control mAb shows no immunostaining. Fig 7C: Virtually all lymphocytes in native CA lesions (as well as many macrophages and foam cells) were $CD4^+$, suggesting that the $CD40L^+$ lymphocytes are $CD4^+$ T cells. Fig 7D: $CD8^+$ T cells were rare in intimal plaques of native CA. (Figs 7A, 7B x1000, Figs 7C, 7D x400)

Figures 8A-8C: Deep intimal lymphoid aggregates in TCAD labelled with anti-CD40L mAb (Fig 8A), control mAb (Fig 8B) and anti-CD4 mAb (Fig 8C). Fig 8A: Most of the $CD40L^+$ cells in TCAD (arrows) were found in lymphoid aggregates within the intima and away from the endothelial surface. Fig 8B: The irrelevant isotype matched control mAb shows no cellular staining in such intimal lymphoid aggregates. Fig 8C: The same intimal lymphoid aggregate as above contains almost exclusively $CD4^+$ T cells suggesting that CD40L is expressed on $CD4^+$ T cells in these lesions. (Figs 8A-8C x400).

Figures 9A-9B: Focus of endothelitis in TCAD stained with anti-CD8 (Fig 9A) and anti-CD40L (Fig 9B) mAbs. Fig 9A: $CD8^+$ T cells attached to the luminal endothelial cells in TCAD characteristic for endothelitis. Most of the $CD8^+$ T cells were present in foci of endothelitis, whereas they were rarely present in intimal lymphoid aggregates away from the endothelial surface. Fig 9B: Inflammatory cells in foci of endothelitis are $CD40L^-$. Similarly, CD40L expression was not detected on endothelial cells. (Figs 9A-9B x400)

Figures 10A-10B: Fig 10A: Double immunolabelling of intimal lesion of native CA with anti-CD40 mAb (brown) and anti-CD68 mAb (red), a marker for macrophages. The central cluster of cells (arrows) shows strong staining for both CD40 and CD68. Fig 10B: Double immunolabelling of TCAD with anti-CD40 mAb (brown) and anti-smooth muscle actin mAb (red) demonstrates CD40+ smooth muscle cells

(arrows). CD40 reactivity is confined to intimal smooth muscle cells (arrows), whereas medial myocytes were CD40-. (Figs 10A-B x400)

Figures 11A-11D: Serial sections of native CA demonstrating intimal neovascularization and stained with anti-CD34 (Fig 11 A), anti-CD40 (Fig 11B), anti-ICAM-1 (Fig 11C), and anti-VCAM-1 (Fig 11D) mAbs. Fig 11A: Endothelial cells of intimal neovessels highlighted by CD34 staining. Fig 11B: Intimal neovascular endothelial cells strongly express CD40. The adjacent inflammatory cells also label for CD40. Figs 11C, 11D: Foci of neovascularization also showed strong endothelial reactivity for ICAM-1 (Fig 11C), and VCAM-1 (Fig 11D). (Figs 11A-11D x400).

Figures 12A-12C: Double immunolabelling of actively inflamed intimal lesion of native CA with anti-CD40 mAb (brown) and adhesion molecules (red) anti-ICAM-1 mAb (Fig 12A), anti-VCAM-1 mAb (Fig 12B) and irrelevant control mAb (Fig 12C). Fig 12A: Virtually all $CD40^+$ (brown) cells, predominantly macrophages (long arrows), and intimal myocytes (short arrows), are strongly reactive for ICAM-1 (red). Fig 12B: A large number of $CD40^+$ (brown) inflammatory cells and intimal myocytes (arrows) are also reactive for VCAM-1 (red). Fig 12C: Same intimal lesion double labelled for CD40 (brown) and irrelevant isotype matched control Ab substituted for anti-ICAM-1 and anti-VCAM-1 mAbs (red). Only brown and no red staining is discerned indicating absence of interference of detection techniques for the sequentially applied anti-CD40 and anti-ICAM or anti-VCAM mAbs (see Materials and Methods). (Figs 12A-C x400).

Figure 13: Double immunolabelling of intimal lesion of native CA with anti-p65 mAb labelling activated NF-kB (brown) and CD40 (red). Activated NF-κB was exclusively

discerned in nuclei of $CD40^+$ cells (arrows), most of which are macrophages. (x400).

Detailed Description

This invention provides a method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the cell surface, comprising contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells. In one embodiment of this invention the agent is capable of inhibiting any interaction between CD40 ligand and CD40. "Interaction between CD40 ligand and CD40 on the cells" refers to one or more aspects, functional or structural, of a CD40-CD40 ligand interrelationship. Therefore, in one embodiment, an agent which inhibits interaction may competitively bind to CD40 ligand in such a way to block or diminish the binding of CD40 ligand to cellular CD40. In another embodiment an agent which inhibits interaction may associate with CD40 or CD40 ligand in a manner which does not inhibit binding of CD40 ligand to cellular CD40, but which influences the cellular response to the CD40 ligation, such as by altering the turnover rate of the cellular CD40 or the CD40-agent complex, by altering binding kinetics of CD40 with CD40 ligand, or by altering the rate or extent of cellular activation in response to CD40 ligation.

In specific embodiments the CD40-bearing smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, bronchial smooth muscle cells, aortic smooth muscle cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells. In more specific embodiments the gastrointestinal smooth muscle cells are esophageal, stomach, or intestinal smooth muscle cells, including smooth muscle cells of the small intestine or the large intestine (bowel).

In an embodiment of this invention the agent inhibits binding of CD40 ligand to CD40 on the cells.

In an embodiment of this invention the agent is a protein.

In another embodiment of this invention the agent is a nonprotein. As used herein the term nonprotein includes any and all compounds or agents which encompass elements other than simple or conjugated polypeptide chains. This includes elements such as amino acids having non-peptide linkages; nonprotein amino acids such as ß, γ, or δ amino acids, amino acids in D configuration, or other nonprotein amino acids including homocysteine, homoserine, citrulline, ornithine, γ-aminobutyric acid, canavanine, djenkolic acid, or ß-cyanoalanine; monosaccharides, polysaccharides, or carbohydrate moieties; fatty acids or lipid moieties; nucleotide moieties, mineral moieties; or other nonprotein elements.

In another embodiment of this invention, the agent is a peptidomimetic compound. The peptidomimetic compound may be at least partially unnatural. The peptidomimetic compound may be a small molecule mimic. The compound may have increased stability, efficacy, potency and bioavailability by virtue of the mimic. Further, the compound may have decreased toxicity. The peptidomimetic compound may have enhanced mucosal intestinal permeability. The compound may be synthetically prepared. The compound of the present invention may include L-,D- or unnatural amino acids, alpha, alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid (an isoelectronic analog of alanine). The peptide backbone of the compound may have at least one bond replaced with PSI-[CH=CH] (Kempf et al. (1991) *Intl. J. Peptide and Prot. Res.* **38**, 237-241). The compound may

further include trifluorotyrosine, p-Cl-phenylalanine, p-Br-phenylalanine, poly-L-propargylglycine, poly-D,L-allyl glycine, or poly-L-allyl glycine.

In another embodiment of the present invention, the peptidomimetic compound having the biological activity of inhibiting interaction between CD40 ligand and CD40 on cells may have a bond, a peptide backbone or an amino acid component replaced with a suitable mimic. Examples of unnatural amino acids which may be suitable amino acid mimics include ß-alanine, L-α-amino butyric acid, L-γ-amino butyric acid, L-α-amino isobutyric acid, L-ε-amino caproic acid, 7-amino heptanoic acid, L-aspartic acid, L-glutamic acid, cysteine (acetamindomethyl), N-ε-Boc-N-α-CBZ-L-lysine, N-ε-Boc-N-α-Fmoc-L-lysine, L-methionine sulfone, L-norleucine, L-norvaline, N-α-Boc-N-δCBZ-L-ornithine, N-δ-Boc-N-α-CBZ-L-ornithine, Boc-p-nitro-L-phenylalanine, Boc-hydroxyproline, Boc-L-thioproline. (Blondelle, S.E. et al., (1994) *Antimicrobial Agents and Chemotherapy* **38**, 2280-2286.; Pinilla, C., et al. (1995) *Peptide Science* **37**, 221-240).

In a specific embodiment the protein comprises an antibody or portion thereof capable of inhibiting interaction between CD40 ligand and CD40 on the cells. The antibody is a monoclonal or polyclonal antibody. In a more specific embodiment the monoclonal antibody specifically binds to the epitope to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds. An example of such a monoclonal antibody is monoclonal antibody 5c8 (ATCC Accession No. HB 10916). In another embodiment, the antibody specifically binds to CD40. One example of an anti-CD40 antibody is the monoclonal mouse anti-human CD40, available from Genzyme Customer Service (Product 80-3702-01, Cambridge, MA). In other embodiments the monoclonal antibody is a chimeric antibody, a primatized antibody, a humanized antibody, or

an antibody which includes a CDR region from a first human and an antibody scaffold from a second human.

The meaning of "chimeric", "primatized" and "humanized" antibody and methods of producing them are well known to those of skill in the art. See, for example, PCT International Publication No. WO 90/07861, published July 26, 1990 (Queen, et al.); and Queen, et al. Proc. Nat'l Acad. Sci.-USA (1989) 86: 10029. Methods of making primatized antibodies are disclosed, for example, in PCT International publication No. WO __/02108, corresponding to International Application No. PCT/US92/06194 (Idec Pharmaceuticals); and in Newman, et al., Biotechnology (1992) 10:1455-1460, which are hereby incorporated by reference into this application.

Generally, a humanized antibody is an antibody comprising one or more complementarity determining regions (CDRs) of a non-human antibody functionally joined to human framework region segments. Additional residues associated with the non-human antibody can optionally be present. Typically, at least one heavy chain or one light chain comprises non-human CDRs. Typically, the non-human CDRs are mouse CDRs. Generally, a primatized antibody is an antibody comprising one or more complementarity determining regions (CDRs) of an antibody of a species other than a non-human primate, functionally joined to framework region segments of a non-human primate. Additional residues associated with the species from which the CDR is derived can optionally be present. Typically, at least one heavy chain or one light chain comprises CDRs of the species which is not a nonhuman primate. Typically, the CDRs are human CDRs. Generally, a chimeric antibody is an antibody whose light and/or heavy chains contain regions from different species. For example one or more variable (V) region segments of one species may be joined to one or more constant (C) region

segments of another species. Typically, a chimeric antibody contains variable region segments of a mouse joined to human constant region segments, although other mammalian species may be used.

Monoclonal antibody 5c8 is produced by a hybridoma cell which was deposited on November 14, 1991 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The hybridoma was accorded ATCC Accession Number HB 10916.

In a specific embodiment the portion of the antibody comprises a complementarity determining region or variable region of a light or heavy chain. In another specific embodiment the portion of the antibody comprises a complementarity determining region or a variable region. In another specific embodiment the portion of the antibody comprises a Fab or a single chain antibody. A single chain antibody is made up of variable regions linked by protein spacers in a single protein chain.

In another embodiment the protein comprises soluble extracellular region of CD40 ligand, or portion thereof, or variant thereof, capable of inhibiting interaction between CD40 ligand and CD40 on the cells; or soluble extracellular region of CD40, or portion thereof, or variant thereof, capable of inhibiting interaction between CD40 ligand and CD40 on the cells. In a specific embodiment the soluble extracellular region of CD40 ligand or CD40 is a monomer. In another embodiment the soluble extracellular region of CD40 is an oligomer.

Variants can differ from naturally occurring CD40 or CD40 ligand in amino acid sequence or in ways that do not

involve sequence, or both. Variants in amino acid sequence are produced when one or more amino acids in naturally occurring CD40 or CD40 ligand is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring CD40 or CD40 ligand, or biologically active fragments of naturally occurring CD40 or CD40 ligand, whose sequences differ from the wild type sequence by one or more conservative amino acid substitutions, which typically have minimal influence on the secondary structure and hydrophobic nature of the protein or peptide. Variants may also have sequences which differ by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the CD40 or CD40 ligand biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid,glutamic acid; asparagine,glutamine; serine,threonine; lysine, arginine; and phenylalanine,tyrosine. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Other conservative substitutions can be taken from Table 1, and yet others are described by Dayhoff in the Atlas of Protein Sequence and Structure (1988).

Table 1: Conservative Amino Acid Replacements

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly,beta-ALa, L-Cys,D-Cys
Arginine	R	D-Arg, Lys,homo-Arg, D-homo-Arg, Met,D-Met, Ile, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn,Asp,D-Asp,Glu,D-Glu, Gln,D-Gln
Aspartic Acid	D	D-Asp,D-Asn,Asn, Glu,D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys,Met,D-Met,Thr, D-Thr
Glutamine	Q	D-Gln,Asn, D-Asn,Glu,D-Glu,Asp, D-Asp
Glutamic Acid	E	D-Glu,D-Asp,Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala,Pro, D-Pro, Beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys,Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val, Norleu
Phenylalanine	F	D-Phe,Tyr, D-Thr,L-Dopa,His,D-His, Trp, D-Trp, Trans 3,4 or 5-phenylproline, cis 3,4 or 5 phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid

Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O) D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr,Phe, D-Phe, L-Dopa, His,D-His
Valine	V	D-Val, Leu,D-Leu,Ile,D-Ile, Met, D-Met

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D- instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e.g., U.S. Patent 5,219,990.

The peptides of this invention may also be modified by various changes such as insertions, deletions and substitutions, either conservative or nonconservative where such changes might provide for certain advantages in their use.

In other embodiments, variants with amino acid substitutions which are less conservative may also result in desired derivatives, e.g., by causing changes in charge, conformation and other biological properties. Such substitutions would include for example, substitution of hydrophilic residue for a hydrophobic residue, substitution of a cysteine or proline for

another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or substitution of a residue having a net positive charge for a residue having a net negative charge. When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of the desired characteristics.

Variants within the scope of the invention include proteins and peptides with amino acid sequences having at least eighty percent homology with the extracellular region of CD40 or the extracellular region of CD40 ligand. More preferably the sequence homology is at least ninety percent, or at least ninety-five percent.

Just as it is possible to replace substituents of the scaffold, it is also possible to substitute functional groups which decorate the scaffold with groups characterized by similar features. These substitutions will initially be conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group. Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatization of portions of naturally occurring CD40 or CD40 ligand, as well as changes in acetylation, methylation, phosphorylation, carboxylation or glycolsylation.

In a further embodiment the protein, including the extracellular region of CD40 ligand and CD40, is modified by chemical modifications in which activity is preserved. For example, the proteins may be amidated, sulfated, singly or multiply halogenated, alkylated, carboxylated, or phosphorylated. The protein may also be singly or multiply acylated, such as with an acetyl group, with a farnesyl moiety, or with a fatty acid, which may be

saturated, monounsaturated or polyunsaturated. The fatty acid may also be singly or multiply fluorinated. The invention also includes methionine analogs of the protein, for example the methionine sulfone and methionine sulfoxide analogs. The invention also includes salts of the proteins, such as ammonium salts, including alkyl or aryl ammonium salts, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, thiosulfate, carbonate, bicarbonate, benzoate, sulfonate, thiosulfonate, mesylate, ethyl sulfonate and benzensulfonate salts.

The soluble, monomeric CD40-L protein can comprise all or part of the extracellular region of CD40-L. The extracellular region of CD40-L contains the domain that binds to CD40. Thus, soluble CD40-L can inhibit the interaction between CD40L and the CD40-bearing cell. This invention contemplates that sCD40-L may constitute the entire extracellular region of CD40-L, or a fragment or derivative containing the domain that binds to CD40.

Soluble CD40 protein (sCD40) comprises the extracellular region of CD40. sCD40 inhibits the interaction between CD40L and CD40-bearing cells. sCD40 may be in monomeric or oligomeric form.

In another embodiment of this invention the protein comprising soluble extracellular region of CD40 or portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof. In a specific embodiment the Fc region is capable of binding to protein A or protein G. In another embodiment the Fc region comprises IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgA_1, IgA_2, IgM, IgD, or IgE.

The soluble CD40/Fc fusion protein can be prepared using conventional techniques of enzymes cutting and ligation

of fragments from desired sequences. Suitable Fc regions for the fusion protein are Fc regions that can bind to protein A or protein G, or that are capable of recognition by an antibody that can be used in purification or detection of a fusion protein comprising the Fc region. For example, the Fc region may include the Fc region of human IgG_1 or murine IgG_1. This invention also provides a nucleic acid molecule which encodes the CD40/Fc fusion protein.

The method of creating soluble forms of membrane molecules by recombinant means, in which sequences encoding the transmembrane and cytoplasmic domains are deleted, is well known. See generally Hammonds et al., U.S. Patent No. 5,057,417. In addition, methods of preparing sCD40 and CD40/Fc fusion protein are well-known. See, e.g., PCT International Publication No. WO 93/08207; Fanslow et al., "Soluble Forms of CD40 Inhibit Biologic Responses of Human B Cells, "J. Immunol., vol. 149, pp.655-60 (July 1992).

In an embodiment of this invention, the agent is selected by a screening method.

In a specific embodiment the agent is selected by a screening method, which comprises isolating a sample of cells; culturing the sample under conditions permitting activation of CD40-bearing cells; contacting the sample with cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, effective to activate the CD40-bearing cells; contacting the sample with an amount of the agent effective to inhibit activation of the CD40-bearing cells if the agent is capable of inhibiting

activation of the CD40-bearing cells; and determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, or with the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, activate the CD40-bearing cells in the presence of the agent. The cell sample may be isolated from diverse tissues, including cell lines in culture or cells isolated from an animal, such as dispersed cells from a solid tissue, cells derived from a bone marrow bipsy, or cells isolated from a body fluid such as blood or lymphatic fluid.

In another specific embodiment the agent (molecule) is selected based on a three-dimensional structure of soluble extracellular region of CD40 ligand or portion thereof capable of inhibiting interaction between CD40 ligand and CD40 on the cells. The agent may be selected from a library of known agents, modified from a known agent based on the three-dimensional structure, or designed and synthesized de novo based on the three-dimensional structure. In specific embodiments the agent (molecule) is designed by structure optimization of a lead inhibitory agent based on a three-dimensional structure of a complex of the soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitory agent. A lead inhibitory agent is a molecule which has been identified which, when it is contacted with CD40 ligand, binds to and complexes with the soluble extracellular region of CD40 ligand, CD40, or portion thereof, thereby decreasing the ability of the complexed or bound CD40 ligand or CD40 ligand portion to activate CD40-bearing cells. In another embodiment, a lead inhibitory agent may act by interacting with either the extracellular region of CD40 ligand, CD40, or in a tertiary complex with both a portion of CD40 ligand and

CD40, decreasing the ability of the complexed CD40 ligand-CD40 to activate the CD40-bearing cells. In the methods of the invention, the CD40 ligand may be either soluble or bound to cells such as activated T cells, and may be either full length native CD40 ligand or portions thereof. Decreased ability to activate CD40-bearing cells may be measured in different ways. One way it may be measured is by showing that CD40 ligand, in the presence of inhibitor, causes a lesser degree of activation of CD40-bearing cells, as compared to treatment of the cells with a similar amount of CD40 ligand without inhibitor under similar conditions. Decreased ability to activate CD40-bearing cells may also be indicated by a higher concentration of inhibitor-CD40 ligand complex being required to produce a similar degree of activation of CD40-bearing cells under similar conditions, as compared to unbound CD40 ligand. At the extreme, the inhibitor-contacted CD40 ligand may be unable to activate CD40-bearing cells at concentrations and under conditions which allow activation of these cells by unbound CD40 ligand or a given portion thereof.

The agent (molecule) can be selected by a computational screening method using the crystal structure of a soluble fragment of the extracellular domain of human CD40L containing residues Gly116-Leu261 (sCD40L(116-261)).

The crystal structure to be used with the screening method has been determined at 2 Å resolution by the method of molecular replacement. In brief, a soluble fragment of the extracellular domain of human CD40 ligand containing amino acid residues Gly 116 to the c-terminal residue Leu 261 was first produced in soluble form, then purified and crystallized. The crystals were used to collect diffraction data. Molecular replacement and refinement were done with the XPLOR program package and QUANTA (Molecular Simulations, Inc.) Software. In

particular, a 3-dimensional model of human sCD40L was constructed using the murine CD40L model using QUANTA protein homology modeling software. This model was used as a probe for crystallographic analysis calculations and refined using XPLOR. This method of determining the crystal structure of sCD40L is described in more detail in Karpusas et al., "2 Å crystal structure of an extracellular fragment of human CD40 ligand," Structure (October 1995) 3(10):1031-1039. The atomic coordinates of sCD40L(116-261) are provided in Figures 2A-Y. The screening method for selecting an agent includes computational drug design and iterative structure optimization, as described below.

The agent may be an inhibitor selected using computational drug design. Using this method, the sCD40L crystal structure coordinates are used as an input for a computer program, such as DOCK, which outputs a list of molecular structures that are expected to bind to CD40L. Use of such computer programs is well-known. See, e.g., Kuntz, "Structure-Based Strategies for drug design and discovery," Science, vol. 257, p. 1078 (1992). The list of molecular structures can then be screened by biochemical assays for CD40L binding. Competition-type biochemical assays, which are well known, can be used. See, e.g., Bajorath et al., "Identification of residues of CD40 and its ligand which are critical for the receptor-ligand interaction," Biochemistry, 34, p. 1833 (1995). The structures that are found to bind to CD40L can thus be used as agents for the present invention. The agent may also be a modified or designed molecule, determined by interactive cycles of structure optimization. Using this approach, a small molecule inhibitor of CD40L found using the above computational approach or other approach can be co-crystallized with sCD40L and the crystal structure of the complex solved by molecular replacement. The information revealed through molecular replacement

can be used to optimize the structure of the inhibitors by clarifying how the molecules interact with CD40L. The molecule may be modified to improve its physiochemical properties, including specificity and affinity for CD40L.

In an embodiment of this invention the agent is a small molecule. As used herein a small molecule is a compound having a molecular weight between 20 Da and $1x10^6$ Da, preferably from 50 Da to 2 kDa.

This invention also provides a method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells in the subject.

In specific embodiments the CD40-bearing smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, bronchial smooth muscle cells, aortic smooth muscle cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells. In more specific embodiments the gastrointestinal smooth muscle cells are esophageal, stomachic, or intestinal smooth muscle cells, including smooth muscle cells of the small intestine or large intestine (bowel).

In an embodiment of this invention the agent inhibits binding of CD40 ligand to CD40 on the cells.

In an embodiment of this invention the agent is a protein. In another embodiment of this invention the agent is a nonprotein.

In a specific embodiment the protein comprises an

antibody or portion thereof capable of inhibiting interaction between CD40 ligand and CD40 on the cells. The antibody is a monoclonal or polyclonal antibody. In a more specific embodiment the monoclonal antibody specifically binds to the epitope to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds. An example of such a monoclonal antibody is monoclonal antibody 5c8 (ATCC Accession No. HB 10916). In other embodiments the monoclonal antibody is a chimeric antibody or a humanized antibody.

In a specific embodiment the portion of the antibody comprises a complementarity determining region or variable region of a light or heavy chain. In another specific embodiment the portion of the antibody comprises a complementarity determining region or a variable region. In another specific embodiment the portion of the antibody comprises a Fab or a single chain antibody.

In another embodiment the protein comprises soluble extracellular region of CD40 ligand or portion thereof capable of inhibiting interaction between CD40 ligand and CD40 on the cells; or soluble extracellular region of CD40 or portion thereof capable of inhibiting interaction between CD40 ligand and CD40 on the cells. In a specific embodiment the soluble extracellular region of CD40 ligand or CD40 is a monomer. In another embodiment the soluble extracellular region of CD40 is an oligomer.

In another embodiment of this invention the protein comprising soluble extracellular region of CD40 or portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof. In a specific embodiment the Fc region is capable of binding to protein A or protein G. In another specific embodiment the Fc region comprises IgG, IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgA_1, IgA_2, IgM, IgD, or IgE.

When administered, proteins are often cleared rapidly from the circulation and may therefore elicit relatively short-lived pharmacological activity. Consequently, frequent injections of relatively large doses of bioactive proteins may by required to sustain therapeutic efficacy. Proteins modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified proteins (Abuchowski et al., In: "Enzymes as Drugs", Holcenberg et al., eds. Wiley-Interscience, New York, NY, 367-383 (1981; Anderson, W.F. (1992) Human Gene Therapy. Science *256*:808-813.; Newmark et al., (1982) J. Appl. Biochem. 4:185-189; and Katre et al., Proc. Natl. Acad. Sci. USA 84:1487-1491 (1987)). Such modifications may also increase the protein's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the protein, and greatly reduce the immunogenicity and antigenicity of the protein. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-protein adducts less frequently or in lower doses than with the unmodified protein.

Attachment of polyethylene glycol (PEG) to proteins is particularly useful because PEG has very low toxicity in mammals (Carpenter et al., 1971). For example, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. A second advantage afforded by the conjugation of PEG is that of effectively reducing the immunogenicity and antigenicity of heterologous proteins. For example, a PEG adduct of a human protein might be useful for the treatment of

disease in other mammalian species without the risk of triggering a severe immune response. In one embodiment of this invention, the protein may be delivered in a microencapsulation device so as to reduce or prevent an host immune response against the protein. The protein may also be delivered microencapsulated in a membrane, such as a liposome.

Polymers such as PEG may be conveniently attached to one or more reactive amino acid residues in a protein such as the alpha-amino group of the aminoterminal amino acid, the epsilon amino groups of lysine side chains, the sulfhydryl groups of cysteine side chains, the carboxyl groups of aspartyl and glutamyl side chains, the alpha-carboxyl group of the carboxy-terminal amino acid, tyrosine side chains, or to activated derivatives of glycosyl chains attached to certain asparagine, serine or threonine residues.

Numerous activated forms of PEG suitable for direct reaction with proteins have been described. Useful PEG reagents for reaction with protein amino groups include active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate. PEG derivatives containing maleimido or haloacetyl groups are useful reagents for the modification of protein free sulfhydryl groups. Likewise, PEG reagents containing amino hydrazine or hydrazide groups are useful for reaction with aldehydes generated by periodate oxidation of carbohydrate groups in proteins.

The subject which can be treated by the above-described methods is an animal. Preferably the animal is a mammal. Examples of mammals which may be treated include, but are not limited to, humans, non-human primates, rodents

(including rats, mice, hamsters and guinea pigs) cow, horse, sheep, goat, pig, dog and cat.

In an embodiment of this invention, the agent is selected by a screening method.

In a specific embodiment the agent is selected by a screening method, which comprises isolating a sample of cells; culturing the sample under conditions permitting activation of CD40-bearing cells; contacting the sample with cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, effective to activate the CD40-bearing cells; contacting the sample with an amount of the agent effective to inhibit activation of the CD40-bearing cells if the agent is capable of inhibiting activation of the CD40-bearing cells; and determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, or with the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession no. HB 10916, activate the CD40-bearing cells in the presence of the agent. The cell sample may be isolated from diverse tissues, including cell lines in culture or cells isolated from an animal, such as dispersed cells from a solid tissue, cells derived from a bone marrow bipsy, or cells isolated from a body fluid such as blood or lymphatic fluid.

In another specific embodiment the molecule (agent) is selected based on a three-dimensional structure of soluble extracellular region of CD40 ligand or portion thereof capable of inhibiting interaction between CD40

ligand and CD40 on the cells. The molecule may be selected from a library of known molecules, modified from a known molecule based on the three-dimensional structure, or designed and synthesized de novo based on the three-dimensional structure. In specific embodiments the agent or molecule is designed by structure optimization of a lead inhibitory agent based on a three-dimensional structure of a complex of the soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitory agent.

Method of Treatment

This invention provides a method of treating, in a subject, a smooth muscle cell-dependent disease, comprising the above-described method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells, which comprises administering to the subject an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells in the subject.

In an embodiment of this invention the smooth muscle cell-dependent disease is a vascular disease. In a specific embodiment the vascular disease is atherosclerosis.

In another embodiment the smooth muscle cell-dependent disease is a gastrointestinal disease. In a specific embodiment the gastrointestinal disease is selected from the group consisting of esophageal dysmotility, inflammatory bowel disease, and scleroderma.

In an embodiment the smooth muscle cell-dependent disease is a bladder disease.

The compounds of this invention may be administered in any manner which is medically acceptable. This may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, or others as well as oral, nasal, ophthalmic, rectal, topical, or inhaled. Sustained release administration is also specifically included in the invention, by such means as depot injections of erodible implants directly applied during surgery.

The compounds are administered at any dose per body weight and any dosage frequency which is medically acceptable. Acceptable dosage includes a range of between about 0.01 and 200 mg/kg subject body weight. A preferred dosage range is between about 0.1 and 50 mg/kg. Particularly preferred is a dose of between about 1 and 30 mg/kg. The dosage is repeated at intervals ranging from each day to every other month. One preferred dosing regimen is to administer a compound of the invention daily for the first three days of treatment, after which the compound is administered every 3 weeks, with each administration being intravenously at 5 or 10 mg/kg body weight. Another preferred regime is to administer a compound of the invention daily intravenously at 5 mg/kg body weight for the first three days of treatment, after which the compound is administered subcutaneously or intramuscularly every week at 10 mg per subject. Another preferred regime is to administer a single dose of the compound of the invention parenterally at 20 mg/kg body weight, followed by administration of the compound subcutaneously or intramuscularly every week at 10 mg per subject.

The compounds of the invention may be administered as a single dosage for certain indications such as preventing

immune response to an antigen to which a subject is exposed for a brief time, such as an exogenous antigen administered on a single day of treatment. Examples of such an antigen would include coadministration of a compound of the invention along with a gene therapy vector, or a therapeutic agent such as an antigenic pharmaceutical or a blood product. In indications where antigen is chronically present, such as in controlling immune reaction to transplanted tissue or to chronically administered antigenic pharmaceuticals, the compounds of the invention are administered at intervals for as long a time as medically indicated, ranging from days or weeks to the life of the subject.

Inflammatory responses are characterized by redness, swelling, heat and pain, as consequences of capillary dilation with edema and migration of phagocytic leukocytes. Inflammation is further defined by Gallin (Chapter 26, Fundamental Immunology, 2d Ed., Raven Press, New York, 1989, pp. 721-733), which is herein incorporated by reference.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Examples 1 and 2 below demonstrate that inflammatory cytokines induce smooth muscle cells to express CD40. Moreover, they demonstrate that CD40L mediated signals regulate smooth muscle cell functions.

EXAMPLE 1

FACS analysis was utilized to investigate if smooth muscle cells express CD40. In 6 well plates human aortic smooth muscle cells were cultured in M199 media supplemented with 25% FCS, 5% human serum, heparin 90 μg/ml, endothelial cell growth factor 15 μg/ml, and 1% penicillin-streptomycin. The media was changed every 2-3 days and when the cells were near confluent they were cultured in the presence or absence of IFN-γ (1000 U/cc), IL-1α (1 ng/cc) or TNF-α (200 U/cc) for 72 hours. The cells were collected by trypsin-EDTA treatment and CD40 expression determined by FACS analysis utilizing anti-CD40 mAb G28.5. The cells were also stained with an isotype negative control mAb and anti-CD54 (ICAM-1) mAb was utilized as a positive control.

Smooth muscle cells do not constitutively express CD40 as demonstrated in **Figure 1A**. However, IFN-γ in contrast to IL-1α or TNF-α, upregulates smooth muscle cell CD40 expression **(Figures 1A, 1B, and 1C)**. These studies demonstrate that IFN-γ upregulates CD40 expression on human aortic smooth muscle cells.

EXAMPLE 2

CD40 expression on smooth muscle cell was examined in situ. Cells found in the media of normal vessels which morphologically resemble smooth muscle cells do not react with anti-CD40 mAb. However, cells which morphologically

resemble smooth muscle cells found within inflammatory lesions in accelerated atherosclerosis associated with transplantation express CD40 in situ. These studies suggest that inflammatory cytokines induce smooth muscle cells to express CD40. Moreover, these studies demonstrate that CD40L-mediated signals regulate smooth muscle cell functions.

EXAMPLE 3

CD40L$^+$CD4$^+$ T Cells and CD40$^+$ Target Cells are Present in Atherosclerosis and Transplant Coronary Artery Disease.

Activated endothelial cells (EC), macrophages (Mac) and CD4$^+$ T cells are present early in the lesions of coronary atherosclerosis (CA) and cardiac transplant atherosclerosis (TA). Because CD40L is an activation-induced CD4$^+$ T cell surface molecule that delivers contact-dependent activating signals to CD40$^+$ target cells including EC (upregulated ICAM, VCAM and E-selectin expression) and Mac (induces NO, TNF-α and IL-1 production), we investigated in situ CD40L and CD40 expression in CA (n=5) and TA (n=5). CD40L and CD40 expression was determined utilizing anti-CD40L mAb 5C8, anti-CD40 mAb G28.5 or appropriate control mAbs. Frozen sections of normal coronary arteries (n=3) do not contain T cells and CD40 expression is restricted to EC. In contrast, lesions associated with CA and TA contain CD40L$^+$CD4$^+$ T cells as determined by immunolabelling of serial sections. Additionally, CD40 expression in frozen sections from patients with CA and TA is markedly upregulated on EC, infiltrating mononuclear cells, foam cells and intimal smooth muscle cells (SMC). Two color immunohitochemical analysis of paraffin fixed tissue utilizing SMC (smooth muscle actin) or Mac (HAM-56) specific markers confirm the expression of CD40 on these cells. Interestingly, intimal SMC distant from

inflammatory cells and medial SMC are $CD40^-$, suggesting that local inflammatory mediators upregulate CD40 expression on SMC in vivo. CD40 upregulation and $CD40L^+CD4^+$ T cells are found in all stages of TA and are most marked in early lesions of CA, including fatty streaks. Together, these studies suggest that $CD40L^+$ T cells may interact with $CD40^+$ target cells in CA and TA and contribute to the pathogenesis of these diseases by promoting production of proinflammatory molecules.

Example 4: CD40 is expressed on smooth muscle cells and macrophages in lesions of transplant atherosclerosis.

In situ CD40 expression in native atherosclerosis or transplant associated atherosclerosis was studied by two color immunohistochemical analysis. Double labeling immunohistochemistry studies were performed on coronary arteries that had been fixed in 10% buffered formalin and paraffin embedded. Sections were deparaffinized in xylene, hydrated and endogenous peroxidase quenched with 1/5% H_2O_2 in 80% alcohol. Sections were then digested with 0.01% pepsin in HCl (pH 1.5) for 15 minutes at 37°C. Sections were then rinsed in PBS and incubated with 10% horse serum for 20 minutes to block non-specific staining. Then anti-CD40 staining was detected with the Vector ABC Elite Kit (Vector) sequentially utilizing a biotinylated secondary antibody, avidin-peroxidase complex and 3,3' diaminobenzidine as developer. The presence of CD40 was noted as brown staining. Thereafter, sections were rinsed in PBS and blocked again with 10% horse serum. Sections were then incubated for 1 hour with mAbs specific for smooth muscle cells (smooth muscle actin) or macrophages (HAM 56). The primary antibodies were then conjugated to alkaline phosphatase using an avidin-biotin system (Vector). Vector Red (Vector) was used to detect alkaline phosphatase activity and staining yielded a red reaction. Hence, double

labeled cells stained brown (CD40) and red (smooth muscle cells or macrophages). To control for interference between the two immunohistochemical procedures used for dual labeling analysis, serial sections of each specimen were also stained either for CD40, smooth muscle actin or HAM 56. See **Figures 3A** and **3B**. Control sections showed the same distribution of immunoreactivity for each of the primary mAbs as the double stained sections.

EXAMPLE 5: The Distribution Of CD40L And CD40 In Native Coronary Atherosclerosis And Transplant Associated Coronary Artery Disease: Correlation Of CD40 Expression With The Presence Of Intercellular Adhesion Molecules, Activated NF-kB And Presence Of T Lymphocytes.

T cells play roles in the pathogenesis of native coronary atherosclerosis (CA) and transplant associated coronary artery disease (TCAD), however the mechanisms by which T cells interact with other cells in these lesions are not fully known. CD40L is an activation-induced CD4+ T cell surface molecule that interacts with CD40+ target cells, including macrophages and endothelial cells, and induces the production of proinflammatory molecules, including ICAM-1 and VCAM-1. Moreover, ligation of CD40 is known to activate the transcription factor NF-kB. To investigate whether CD40L-CD40 interactions may play roles in the pathogenesis of CA or TCAD immunohistochemical studies were performed of CD40L and CD40 expression on frozen sections of coronary arteries obtained from cardiac allograft recipients with CA (n=10) or TCAD (n=9). Utilizing two different anti-CD40L mAb it was found that CD40L expression was restricted to infiltrating lymphocytes in CA and TCAD. CD40 expression was markedly upregulated on intimal endothelial cells, foam cells, macrophages and smooth muscle cells in both diseases. Dual immunolabelling demonstrated many CD40+ cells co-expressed ICAM-1, VCAM-1 or the activated form

of NF-kB. The extent of CD40, ICAM-1 and VCAM-1 expression showed statistical significant correlation with the severity of disease and the amount of intimal lymphocytes. Together these studies demonstrate the presence of activated CD40L+ and $CD40^{+}$ cells in both CA and TCAD lesions and suggest that CD40L mediated interactions with CD40+ macrophages, foam cells, smooth muscle cells and/or endothelial cells may contribute to the pathogenesis of these diseases.

Several lines of evidence indicate that cell-mediated immune mechanisms contribute to the inflammatory lesions (1-4) characteristic of native coronary atherosclerosis (CA) (5-10) and transplant-associated coronary artery disease (TCAD) (11-13). For example, infiltrating intimal T cells expressing activation markers such as CD25 and MHC Class II molecules are present early in the development of the vascular lesions of both diseases (5, 14). Activated macrophages are commonly found in lesions of both diseases, as are cytokines associated with T cell dependent immune responses, including IFN-γ, IL-1 and TNF-α (5-17). As further evidence that T cells may play pathogenic roles in CA, $CD4^{+}$ T cell clones have been isolated from human fibroatheromatous CA plaques that proliferate and secrete IFN-γ when presented with oxidized LDL (18), a major constituent of the lesions of both native CA and TCAD (1, 19, 20). Furthermore, hyperlipidemia induced atherosclerotic lesions are reduced in mice treated with anti-CD4 mAbs (21). Similarly, vascular lesions of TCAD are significantly ameliorated when allografts were placed in strains of mice genetically deficient in T cells (13) or treated with anti-CD413 or anti-IFN-γ mAbs (22). Together these data strongly suggest that T cells and T cell-derived effector molecules are involved in the pathogenesis of these diseases (9, 23, 24).

CD40L is a 30-33 kDa MW surface molecule expressed on activated $CD4^+$ T cells which delivers contact-dependent signals to $CD40^+$ target cells, such as B cells (25-29). CD40L mediated signals are critically important in the development of T cell dependent humoral immune responses in vitro and in vivo (30). CD40L-CD40 interactions are now known to also play roles in cell mediated immune responses in vitro and in vivo (31, 32). Interestingly, macrophages and endothelial cells, cell types known to participate in the pathogenesis of CA and TCAD, also express CD40 (33-37). Moreover, ligation of CD40 on macrophages and endothelial cells in vitro induces the production of molecules that enhance immune responses and/or have pro-inflammatory effects. For example, CD40L-CD40 interactions upregulate expression of MHC Class II and the costimulatory molecule CD86 on macrophages in vitro (38). Furthermore, ligation of CD40 on macrophages induces the production of cytokines (TNF-α, IL-1β, IL-12) , chemokines (IL-8, MIP-1α), nitric oxide (NO) via induction of NO synthese 2, the procoagulant protein tissue factor and matrix metalloproteinases (33, 34, 39-42). CD40L-CD40 interactions upregulate intercellular adhesion molecules CD54 (ICAM-1), CD106 (VCAM-1) and CD62E (E-selectin) on endothelial cells (35-37). Many of the effects of CD40 ligation are dependent on activation of the transcription factor NF-κB (43-45).

Together these findings suggest the notion that ligation of CD40 on a variety of target cells may augment $CD4^+$ T cell mediated inflammatory reaction in vivo. In support of this hypothesis, CD40 expression is upregulated in the kidneys of patients with lupus glomerulonephritis, IgA nephropathy and $ANCA^+$ glomerulonephritis and in the skin of patients with psoriasis (35, 46). Moreover, $CD40L^+$ T cells infiltrate the kidneys of patients with inflammatory renal diseases (46). Because interactions

of T cells with macrophages, endothelial cells and possibly other cells play roles in the pathogenesis of CA and TCAD, in the current study the expression of CD40L and CD40 in these two diseases is investigated using immunohistochemistry. CD40L is expressed on T cells and CD40 expression is upregulated on endothelial cells, smooth muscle cells, macrophages and "foam" cells in the intimal lesions of both diseases. Moreover, using double immunostaining it is found that many $CD40^+$ cells in these lesions co-express CD54, CD106 and the activated form of NF-κB.

METHODS: HUMAN CORONARY ARTERIES

Segments from the main left coronary artery or the proximal portion of the left anterior descending artery were obtained from the explanted hearts of 23 cardiac allograft recipients. Nine patients underwent retransplantation because they had developed severe transplant-associated coronary artery disease (TCAD). In these patients survival of the first allograft had ranged between 38 and 103 months. Ten patients received cardiac allografts because they had developed severe coronary artery disease and ischemic cardiomyopathy. Control coronary arteries without atherosclerotic changes were obtained from explanted hearts of 4 patients; 3 had idiopathic cardiomyopathy, one a cardiac sarcoma. Portions of each vessel were snap frozen in isopentane at -80°C and serial sections were cut on a cryostat (Reichert Histostat) at 4 mm thickness. Sections were mounted on sialin coated slides, air dried, fixed in cold acetone for 1 minute, in a 1:1 mixture of cold acetone/chloroform for an additional 7 minutes and stored at -80°C. One section from each coronary artery was fixed in 10% formalin and stained with hematoxylin and eosin for histologic evaluation.

PRIMARY ANTIBODIES

Anti-CD40 hybridoma G28.5 (IgG1) was purchased from American Type Culture Collection (Rockville, MD). Anti-CD40L mAb 5C8 (IgG2a) was generated as previously described (28). Both G28.5 and 5C8 mAbs were purified from ascites utilizing a protein G column (Pharmacia, Piscataway, NJ). An additional anti-CD40L mAb (IgG1) was purchased from Calbiochem (San Diego, CA). An IgM anti-CD40 mAb was obtained from Caltag (Burlingame, CA) and was used for dual immunostaining studies. Monoclonal Abs to CD3, CD4, CD8, CD34, CD68 (Novocastra, Burlingham, CA, all IgG1) and smooth muscle actin (SMA) (DAKO, Carpinteria, CA, IgG2a), were used to distinguish among the various cell types of intimal plaques, including T cells (CD3, CD4 or CD8), endothelial cells (CD34), macrophages (CD68) and smooth muscle cells (SMA). Anti-ICAM-1 (IgG1) and anti-VCAM-1 (IgG1) mAbs were purchased from CHEMICON™ (Temecula, CA). The distribution of activated NF-κB was demonstrated with p65mAb (IgG3) (BOEHRINGER MANNHEIM™) which binds to an epitope on the p65 subunit of NF-κB blocked by IκB and therefore only accessible when NF-κB is activated by dissociation of IκB(47). Isotype control mAb (Mopec 21, 22) were obtained from SIGMA™ (St. Louis, MO).

IMMUNOHISTOCHEMISTRY

Frozen sections were washed in phosphate buffered saline (PBS) and endogenous peroxidase was quenched in 0.5% hydrogen peroxide. Sections were "blocked" with 10% goat serum and aggregated human Ig (80 mg/ml) in PBS and then were incubated for one hour with the indicated primary mAb or the respective control mAb. Frozen sections of tonsils with follicular hyperplasia were used as positive controls to determine the optimal dilution of each mAb. Primary mAb bound to target antigen was linked to biotin labelled isotype specific goat anti-mouse IgG1, IgG2a,

IgG3 or IgM (Fisher Scientific, Pittsburgh, PA), which was then conjugated to avidin-biotin-peroxidase complexes (VECTOR ELITE KIT™,VECTOR™, Burlingham, CA). Peroxidase activity was detected by the chromogen (red) 3-amino-9-ethylcarbazole (AEC,VECTOR™, Burlingham, CA) and the sections were counterstained with Mayer's hematoxylin (SIGMA™, St. Louis, MO).

Double labelling immunohistochemistry was used to identify the cell types expressing CD40 and to determine the distribution of CD40 in relation to ICAM-1, VCAM-1 or activated NF-κB in atherosclerotic lesions. All sections were first immunolabelled with the IgM anti-CD40 mAb. The secondary Ab was a biotinylated goat anti-mouse IgM which was then conjugated to the avidin-biotin-peroxidase complex. The chromogen used to detect the presence of anti-CD40 IgM mAb was 3,3' diaminobenzidine (brown). The sections were then rinsed thoroughly and incubated with a second primary mAb targeting either a cell specific marker for smooth muscle cells (SMA) or macrophages (CD68), leukocyte adhesion molecules (ICAM-1, VCAM-1) or the activated form of NF-κB. All of these second primary mAbs were either IgG1, IgG2a or IgG3 isotypes. The appropriate isotype specific biotinylated secondary antibody was applied and conjugated to an avidin-biotin-alkaline phosphatase complex (VECTOR™, Burlingham, CA). Alkaline phosphatase activity was demonstrated by the chromogen Vector Red (VECTOR™, Burlingham, CA). Interference between the sequentially applied staining procedures was avoided by using different immunoenzymatic techniques (peroxidase vs. alkaline phosphatase) and isotype specific secondary Abs for each target antigen. Furthermore, double labelled control sections were prepared in which one of the two primary mAbs was substituted with an isotype matched control mAb.

Semi-quantitative Analysis of Lesions

The extent of the atherosclerotic lesions in each section was quantitated by the degree of narrowing of the vascular lumen on a scale from 0 to 4 in which 0 indicated no narrowing, 1 less than 25%, 2 less than 50%, 3 less than 90%, and 4 over 90% luminal narrowing. Each coronary artery lesion was also scored for its content of intimal macrophages, smooth muscle cells, foam cells, endothelial cells (neovascularization) 48 and T cells with 0 indicating absence of the respective cell type , 1 rare isolated cells, 2 small collections of cells, 3 focal dense aggregates present, and 4 dense aggregates present throughout the entire plaque. Similarly, the presence of CD40, ICAM-1, and VCAM-1 was scored on a scale from 0 to 4 in which 0 indicates absence of the respective molecule, 1 its presence on rare cells, 2 its presence on less than 50%, 3 on less than 90%, and 4 on more than 90% of all cells (49). Because the expression of CD40L in positive specimens was limited to isolated cells its presence was not amenable to quantitative evaluation.

Statistical Analysis

Differences in histological scores among groups of specimens were analyzed using the non parametric Kruskal Wallis procedure. The association between variables was assessed using Spearman's correlation.

RESULTS: Normal Coronary Arteries

Coronary artery segments from 4 control patients exhibited no intimal thickening or inflammation as demonstrated by H&E staining (Figures 4A-4B). Specifically, macrophages, smooth muscle cells, foam cells or lymphocytes were not present in the intima and no cells were immunoreactive with either anti-CD40L mAb used in this study. CD40 immunoreactivity was present and confined to endothelial cells lining the vascular

lumen of the control arteries (Fig. 4B). VCAM-1 or activated NF-kB was not expressed in the control vessels and ICAM-1 was weakly expressed on rare vascular endothelial cells.

Histology of native CA and TCAD

In 7 of the 10 patients with CA, coronary artery segments revealed prominent fibroatheromatous plaques with eccentric narrowing, acellular lipid-rich cores, cholesterol clefts and overlying fibrous caps. Cellularity of lesions was greatest at the "shoulder" regions which contained macrophages and lymphocytes (Fig. 5A). There were also scattered smooth muscle cells, macrophages, foam cells and foci of neovascularization in the intimal lesions. Plaques from 3 patients with mild, early vascular lesions were eccentric, small, rich in macrophages," foam" cells and lymphocytes.

Coronary artery lesions in the 9 patients with TCAD exhibited circumferential thickening of the intima with marked narrowing of the lumen. (Table 2).

Table 2: Semiquantitative evaluation (scale 0-4) of cell composition in intimal lesion of native coronary atherosclerosis (CA) and transplant coronary artery disease (TCAD) and the immunoreactivity for CD40, ICAM-1, and VCAM-1. Values are expressed as mean + standard deviation.

Intimal Plaque	**Control (n=4)**	**CA (n=10)**	**TCAD (n=9)**
Thickness	0.3±0.5	2.1±0.9*	3.1±0.8*
CD4+ Lymphocytes	0	1.3±0.9*	3.2±0.8*
CD8+ Lymphocytes	0	0.3±0.5	2.6±1.1*
Macrophages (CD68)	0.5±0.6	2.1±0.8*	3.8±0.4*
Foam Cells	0	1.2±0.8*	2.4±1.3*

Smooth Muscle Cells	0.8±1	1.7±0.7	2.9±0.8*
Neovascularization	0	1.8±0.7*	2.6±0.9*
CD40	0.5±0.6	2.2±0.7*	3.3±0.9*
ICAM-1	0.5±0.6	2.3±1.7*	3.6±0.7*
VCAM-1	0.3±0.5	1.7±0.7*	2.9±0.9*

*p,0.05 for CA or TCAD vs. controls by Kruskal - Wallis test

The lesions were composed of concentric layers of smooth muscle cells and interstitial matrix and there was an abundant infiltration with macrophages and lymphocytes along with areas of neovascularization. In 4 coronary arteries lipid-rich atheromatous lesions and "foam" cells were discerned in addition to the concentric layers of smooth muscle cells (Figs. 6A-C). Subendothelial collections of lymphocytes ("endothelitis") and aggregates of lymphocytes in the adventitia were also features noted in TCAD lesions.

Immunohistochemical Analysis of CD40L Expression in CA and TCAD

In marked contrast to normal coronary arteries, which are devoid of infiltrating lymphocytes or CD40L expressing cells, both CA and TCAD lesions contained $CD40L^+$ cells. In native atherosclerosis positive immunostaining for CD40L was confined to a minority of intimal lymphocytes. CD40L staining was usually weak and observed either in small cytoplasmic granules or on the surface of cells (Figs. 7A-D). In native CA most of the intimal lymphocytes were $CD4^+$ T cells; only rare CD8+ T cells were present (Figs. 7A-D). Analysis of serial sections stained with anti-CD4 or anti-CD8 mAbs suggest that the CD40L+ lymphocytes were primarily CD4+ T cells. Endothelial cells, smooth muscle cells, macrophages and "foam" cells did not react with either anti-CD40L mAb

used in this study. No staining was noted with isotype control mAbs.

In TCAD lesions, positive immunostaining for CD40L was also exclusively associated with lymphocytes (Figs. 8A-C). In contrast to CA, both CD8+ and CD4+ T cells were present in TCAD lesions. However, CD8+ T cells were predominately found in subendothelial areas of "endothelitis" (Figs. 9A-B) while CD4+ T cells localized in aggregates deep in the intima adjacent to the internal elastic membrane (Figs. 8A-C) and adventitia of coronary arteries. The expression of CD40L correlated spatially with CD4+ T cells in the intima and adventitia of coronary arteries with TCAD. The number of CD40L+ T cells was higher in TCAD than in native CA lesions. Similar to CA, endothelial cells, smooth muscle cells, macrophages or "foam" cells in TCAD lesions did not react with either anti-CD40L mAb used in this study (Figs. 9A-B). These data indicate that CD40L expressing cells, probably CD4+ T cells, are present in the lesions of native CA and TCAD.

Immunohistochemical Analysis of CD40 Expression in CA and TCAD

In contrast to the weak CD40 expression limited to luminal endothelial cells in normal coronary arteries (Figs. 4A-B), CD40 immunoreactivity was upregulated and broadly distributed in the lesions of native CA (Figs. 5A-B). CD40 expression was noted on endothelial cells, smooth muscle cells, macrophages and "foam" cells. There was a significantly higher mean number of CD40 positive cells in intimal lesions of native CA than in control arteries (2.2+0.7 versus 0.5+0.6, Table 2). Dual immunostaining with macrophage or smooth muscle cell specific markers confirmed that these cells and "foam" cells of both lineages express CD40 (Figs. 10A-B). Interestingly, CD40+ smooth muscle cells were present in

the intima near inflammatory infiltrates, whereas smooth muscle cells in the arterial media did not show positive immunoreactivity for CD40 (Figs. 10A-B). Analysis of serial sections stained with CD40 or the endothelial marker CD34 suggested that endothelial cells lining the intimal neovessels and adventitial vasa vasorum were also strongly CD40+ (Figs. 11A-D).

In arteries from patients with TCAD, the pattern of distribution of CD40 expression was similar to native CA. However, the average score for CD40 immunoreactivity was significantly higher in TCAD than in native CA or control arteries (Table 2). Double immunostaining indicated that intimal smooth muscle cells and macrophages express CD40 (Figs. 10A-B). Moreover, foam cells (Figs. 6A-B) and endothelial cells lining the vascular lumen, intimal neovessels and adventitial vasa vasorum were markedly CD40+. Together, these data demonstrate that endothelial cells, smooth muscle cells and macrophages express CD40 in both native CA and TCAD.

Relationship of CD40 Expression to Intercellular Adhesion Molecules and Activation of NF-kB in CA and TCAD Lesions. Macrophages and endothelial cells in CA and TCAD express intercellular adhesion molecules that regulate the trafficking of leukocytes into the lesion. Because ligation of CD40 induces upregulation of intercellular adhesion molecules and activation of NF-kB on cells in vitro, it was then asked if CD40 expression was associated with the co-expression of intercellular adhesion molecules or NF-kB in CA or TCAD lesions. First it was demonstrated in native CA that luminal endothelial cells manifested focal positive immunostaining for ICAM-1 with rare endothelial cells expressing VCAM-1. In contrast, endothelial cells lining intimal neovessels and adventitial vasa vasorum were strongly positive for ICAM-1 and VCAM-1 (Figs. 11A-D). Intimal smooth muscle

cells, macrophages and "foam" cells were also moderately to strongly positive for ICAM-1 and VCAM-1 (Figs. 12A-C). There was a significant correlation ($p<0.05$) between CD40 scores and those for ICAM-1 ($r=0.85$) and VCAM-1 ($r=0.72$). The number of intimal lymphocytes correlated significantly with the scores for CD40 and the leukocyte adhesion molecules (Table 3).

Table 3: Correlation of scores (0-4) for various cell types of the intimal lesions of CA (n=10) or TCAD (n=9) with scores (0-4) for expression of CD40 and adhesion molecules (ICAM-1, VCAM-1). Values are expressed as the Spearmen correlation coefficient (range -1 to 1, with "0" no correlation and "-1" or "1" perfect correlation).

Cell Type	Group	CD40	ICAM-1	VCAM-1
T-lymphocytes	CA	0.78*	0.77*	0.83**
(CD4+ & CD8+)	TCAD	0.79*	0.87**	0.77*
Macrophages	CA	0.93***	0.84**	0.77*
(CD68+)	TCAD	0.81**	0.68*	0.55
Foam Cells	CA	0.81**	0.68*	0.36
	TCAD	0.44	0.33	0.26
Smooth Muscle	CA	0.72*	0.81**	0.56
Cells (SMA+)	TCAD	0.12	0.38	0.02
Neovessels	CA	0.69*	0.72*	0.53
(CD34+)	TCAD	0.85**	0.87**	0.77*

*$p<0.05$, **$p<0.01$ and ***$p<0.001$ level of sigificance for Spearman Correlation.

Of all listed cell types only the score for intimal lymphocytes correlated significantly with CD40 expression and extent of ICAM-1 and VCAM-1 in intimal plaques in both CA and TCAD suggesting that lymphocytes are involved in the induction of CD40 and adhesion molecules in both diseases. Macrophages and neovascularization also showed significant correlation with CD40 expression in CA and

TCAD.

Double immunostaining of CA lesions with anti-CD40 mAb and anti-ICAM-1 mAb or anti-VCAM-1 mAb showed that CD40 colocalized with these adhesion molecules on many cells (Figs. 12A-C). In addition, activated NF-κB (Fig. 13) was observed in the nuclei of neointimal endothelial cells, macrophages and smooth muscle cells and dual immmunolabeling demonstrated that many CD40+ cells also expressed activated NF-κB.

In TCAD, strongly positive immunostaining for ICAM-1 and VCAM-1 was present on luminal endothelial cells, particularly those near foci of endothelitis. Endothelial cells of intimal neovessels adventitial vasa vasorum were strongly immunoreactive for ICAM-1 and VCAM-1. Scores for immunostaining of the adhesion molecules in TCAD were higher than in CA or normal coronary arteries (Table 2). There was a significant correlation ($p<.05$) between CD40 scores and those for ICAM-1 ($r=0.82$) and VCAM-1 ($r=0.89$). The number of intimal lymphocytes also correlated significantly with the expression of CD40, ICAM-1 and VCAM-1 (Table 3). Similar to CA, two-color immunohistochemistry studies demonstrated that many CD40+ cells in TCAD lesions co-express ICAM-1 or VCAM-1 (Figs. 12A-C). Immunostaining for the activated nuclear form of NF-κB was more widely distributed in TCAD than in native CA. NF-kB positive macrophages and smooth muscle cells were consistently CD40+ (Fig. 13). Together, these studies demonstrate that in lesions of both native CA and TCAD, CD40 is coexpressed on many cells with intercellular adhesion molecules and/or NF-κB.

DISCUSSION

Native atherosclerosis (CA) and transplant related atherosclerosis (TCAD) are inflammatory diseases mediated

by complex interactions between activated T cells, endothelial cells, macrophages and smooth muscle cells (2, 8, 12, 13, 17). T cells are thought to play roles in the pathogenesis of CA and TCAD, however the mechanisms by which they participate in these processes are not fully known (5, 9, 50). Studies have shown that CD40L, an activation induced CD4+ T cell surface molecule, delivers contact-dependent activation signals to CD40 expressing endothelial cells and macrophages that result in the production of pro-inflammatory molecules, such as intercellular adhesion molecules ICAM-1 and VCAM-1 (31, 32, 35-37) and the activation of the transcriptional activating factor NF-κB (43-45, in vitro). Interestingly, TCAD in murine models is at least partly dependent on CD40L-CD40 interactions (51). In the study by Larson and colleagues, anti-CD40L mAb therapy markedly inhibited allogenic hetertopic transplant rejection and partially blocked the associated vasculopathy. Moreover, TCAD in this model was almost completely prevented by administering the combination of anti-CD40L mAb and CTLA4-Ig fusion protein, a molecule that blocks T cell costimulatory pathways (51). It is possible that CD40L-CD40 interactions may participate in the pathogenesis of CA and/or TCAD in humans.

To investigate this hypothesis further immunohistochemical techniques were applied to normal and atherosclerotic coronary arteries to study the expression and cellular distribution of CD40L and CD40. Normal coronary arteries do not contain CD40L expressing cells and CD40 immunoreactivity was restricted to luminal endothelial cells in these vessels. In contrast, CD40L is expressed on lymphocytes in lesions of both native CA and TCAD. It was found that CA lesions contained few CD8+ T cells while TCAD lesions contained CD8+ T cells in close proximity to the luminal endothelium ("endothelitis") and CD4+ T cells deeper in the intima

and adventitia. Based on localization and staining of serial sections with anti-CD4 mAb or anti-CD8 mAb, it was concluded that CD40L+ lymphocytes are most likely CD4+ T cells in the lesions of both diseases. Utilizing two different anti-CD40L mAb it was found that CD40L immunoreactivity was weak and either granular and cytoplasmic or cell surface associated. A similar pattern of CD40L immunoreactivity was noted in a study of CD40L and CD40 expression in glomerulonephritis (46). The weak and frequent cytoplasmic staining pattern of CD40L expression in inflammatory tissues may be related to the transient nature of CD40L expression on activated T cells (27-29) and the fact that engagement of CD40 on target cells induces rapid down-modulation of CD40L by receptor-mediated endocytosis (52) and shedding (53). These regulatory mechanisms probably serve to focus CD40L mediated signaling events to appropriate cognate target cells.

It was found that CD40 expression was markedly upregulated on many cells in the lesions of both diseases. Macrophages and "foam" cells expressing CD40 were particularly prominent in the inflammatory infiltrate of the "shoulder" regions of lipid-rich plaques, which are known to contain dense inflammatory infiltrates (54, 55). CD40 expression was also upregulated on luminal endothelial cells in both diseases and this was particularly prominent in TCAD. Intimal neovessel and adventitial vasa vasorum endothelial cells in both diseases were strongly CD40+. CD40 expressing smooth muscle cells were present in the intima of both CA and TCAD, usually in close proximity to inflammatory infiltrates. Interestingly, smooth muscle cells in the media of the same vessels were CD40-. IFN-γ upregulates CD40 expression on many cells <u>in vitro</u> (33, 35-37, 56) including smooth muscle cells, and this effect is enhanced by cytokines such as IL-1β and TNF-α (36).

Therefore, the marked upregulation of CD40 expression on many cell types in these lesions may be a consequence of cytokine release by lesional T cells, macrophages and other cells. Double immunostaining indicated that many CD40+ cells also co-express intercellular adhesion molecules ICAM-1 and VCAM-1, as well as, the activated form of NF-κB. Together, the current study demonstrates the presence of CD40L+ T cells and activated CD40+ target cells in the vascular lesions of native CA and TCAD.

Early studies showed that CD40 was expressed on some epithelial cell tumors and B cells (57, 58). More recently it has been noted that CD40 is constitutively expressed or inducible on many cell types in vitro (33-37, 56). Furthermore, it is becoming increasingly evident that CD40L-CD40 interactions play key roles in cell-mediated inflammatory reactions in vivo (31, 32). In this regard, recent reports demonstrate in situ CD40L and/or CD40 expression in human inflammatory diseases (35, 46, 59). For example, CD40 expression is upregulated on macrophages infiltrating the brains of patients with multiple sclerosis (59), on dermal endothelial cells and keratinocytes in psoriasis (35), and on many cells in the kidneys of patients with inflammatory glomerulonephritides (46). Moreover, inflammatory infiltrates in the brains of patients with multiple sclerosis (59) and in the kidneys of patients with inflammatory glomerulonephritides 46 contain CD40L+ T cells. It is therefore likely that CD40 expression is upregulated in many inflammatory diseases and represents a molecular mechanism that permits T cells to deliver pro-inflammatory signals to a wide variety of target cells. In this regard, the findings presented herein that CD40 expression is upregulated in CA and TCAD, and that CD40L+ infiltrating T cells are found in lesions, serves as evidence of the hypothesis that immune mediated inflammatory reactions play roles in the pathogenesis of

these diseases (5-7, 9, 18, 21, 23, 50).

Observations regarding CD40L mediated activation of endothelial cells and macrophages in vitro and studies of CD40L-CD40 interactions in the pathogenesis of murine models of TCAD, suggest possible pathogenic roles for CD40L-CD40 interactions in CA and TCAD. For example, CD40L mediated signals upregulate ICAM-1 and VCAM-1 expression on endothelial cells, in vitro (35-37). These intercellular adhesion molecules, which regulate the egress and retention of leukocytes in inflammatory sites, are upregulated on endothelial cells in CA and TCAD and are particularly prominent on intimal neovessel and vasa vasorum endothelial cells (49, 60). Therefore, it is of interest that many CD40+ cells were found in CA and TCAD lesions, and in particular intimal and vasa vasorum endothelial cells, co-express ICAM-1 and/or VCAM-1. Upregulation of ICAM-1 and VCAM-1 is known to be dependent on activation of NF-kB (61). In the present study it was also demonstrated that CD40+ intimal macrophages, smooth muscle cells and endothelial cells express the activated form of NF-kB. These studies suggest that CD40L+ CD4+ T cells may induce upregulation of intercellular adhesion molecules on CD40+ target cells in CA and TCAD, possibly in part by activating NF-kB.

CD40L mediated signals also induce endothelial cells to secrete IL-6 and IL-8 (62) and promotes a procoagulant surface by upregulating tissue factor and down-regulating thrombomodulin expression. With regard to macrophages, CD40L-CD40 interactions induce these cells to secrete proinflammatory cytokines (IL-1α, IL-1ß, IL-6 and TNF-α), chemokines, matrix metalloproteinases and express tissue factor in vitro (33, 34, 38, 41, 42). All these pro-inflammatory molecules probably play roles in the pathogenesis of CA and TCAD (10, 17, 63-66). Ligation of CD40 on macrophages also induces NO production (39, 40).

Interestingly, blocking CD40L-CD40 interactions in murine models of TCAD is associated with down-regulation of iNOS expression and reduction of TCAD lesions (51). It was demonstrated that iNOS is expressed in the lesions of CA (67, 68), cardiac allograft rejection (69, 70) and TCAD (71, 72). CD40L mediated signals may be involved in promoting the production of any of these molecules in CA or TCAD. CD40L-CD40 interactions clearly have pro-inflammatory effects in murine models of TCAD (51), as well as, collagen-induce arthritis (73), lupus-like glomerulonephritis (74) and experimental allergic encephalomyelitis (59).

An investigation (62) of the expression of CD40L and CD40 in human carotid atherosclerosis was carried out. It was found that CD40 was upregulated in lesions and had a broad cellular distribution. CD40L was reported to be widely expressed on smooth muscle cells, endothelial cells and macrophages in the atherosclerotic lesions, whereas in the present study using two different anti-CD40L mAbs, CD40L expression was restricted to T cells. Herein, in situ CD40L expression on macrophages, endothelial cells or smooth muscle cells in either disease was not observed. Similarly, it was found that CD40L immunoreactivity confined to T cells in other inflammatory diseases, including glomerulonephritis (46), rheumatoid arthritis and chronic sinusitis. Additionally, Gerritse et. al. reported that CD40L expression was restricted to CD4+ T cells in multiple sclerosis plaques (59). Discrepancies between results herein and those of Mach and colleagues are currently unclear but may relate to subtle differences in immunohistochemical techniques or in the nature of the lesions.

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(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not Yet Known
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu Ala Ser
1               5                  10                  15

Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr Tyr Thr
            20                  25                  30
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Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln Leu Thr Val
        35                  40                  45

Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr Phe Cys Ser
    50                  55                  60

Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser Leu Cys Leu
65                  70                  75                  80

Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala Ala Asn Thr
                85                  90                  95

His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His Leu Gly Gly
            100                 105                 110

Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn Val Thr Asp
        115                 120                 125

Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu
    130                 135                 140

Lys Leu
145
```

What is claimed is:

1. A method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells, comprising contacting the cells with an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells.

2. The method of claim 1, wherein the smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, aortic smooth muscle cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells.

3. The method of claim 2, wherein the gastrointestinal smooth muscle cells are esophageal smooth muscle cells, stomachic smooth muscle cells, smooth muscle cells of the small intestine, or smooth muscle cells of the large intestine.

4. The method of claim 1, wherein the agent inhibits binding of CD40 ligand to CD40 on the cells.

5. The method of claim 1, wherein the agent is a protein.

6. The method of claim 5, wherein the protein comprises an antibody or portion thereof.

7. The method of claim 6, wherein the antibody is a monoclonal antibody.

8. The method of claim 7, wherein the monoclonal antibody specifically binds to the epitope to which

monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds.

9. The method of claim 8, wherein the monoclonal antibody is monoclonal antibody 5c8 (ATCC Accession No. HB 10916).

10. The method of claim 7, wherein the monoclonal antibody specifically binds to CD40.

11. The method of claim 10, wherein the antibody is humanized, chimeric, or primatized.

12. The method of claim 7, wherein the monoclonal antibody is a chimeric antibody.

13. The method of claim 7, wherein the monoclonal antibody is a humanized antibody.

14. The method of claim 6, wherein the portion of the antibody comprises a complementarity determining region or variable region of a light or heavy chain.

15. The method of claim 6, wherein the portion of the antibody comprises a complementarity determining region or a variable region.

16. The method of claim 15, wherein the portion of the antibody comprises a Fab or a single chain antibody.

17. The method of claim 5, wherein the protein comprises soluble extracellular region of CD40 ligand, or variant thereof including conservative substituents, or portion thereof; or soluble extracellular region of CD40, or variant thereof including conservative substituents, or portion thereof.

18. The method of claim 17, wherein the soluble extracellular region of CD40 ligand or CD40 is a monomer.

19. The method of claim 17, wherein the soluble extracellular region of CD40 is an oligomer.

20. The method of claim 17, wherein the protein comprising soluble extracellular region of CD40 or portion thereof or CD40 ligand or portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof or CD40 ligand or portion thereof.

21. The method of claim 20, wherein the Fc region is capable of binding to protein A or protein G.

22. The method of claim 21, wherein the Fc region comprises IgG, IgA, IgM, IgD, or IgE, or subclasses thereof.

23. The method of claim 22, wherein:
the IgG is IgG_1, IgG_2, IgG_3, or IgG_4; or
the IgA is IgA_1 or IgA_2.

24. The method of claim 1, wherein the agent is nonprotein.

25. The method of claim 1, wherein the agent is selected from a library of known agents.

26. The method of claim 1, wherein the agent is modified from a known agent.

27. The method of claim 26, wherein the modified agent is designed by structure optimization of a lead inhibitory agent based on a three-dimensional

structure of a complex of soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitory agent.

28. The method of claim 1, wherein the agent is selected by a screening method, which comprises:

isolating a sample of cells;

culturing the sample under conditions permitting activation of CD40-bearing cells;

contacting the sample with cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, effective to activate the CD40-bearing cells;

contacting the sample with an amount of the agent effective to inhibit activation of the CD40-bearing cells if the agent is capable of inhibiting activation of the CD40-bearing cells; and

determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, activate the CD40-bearing cells in the presence of the agent.

29. The method of claim 28, wherein the agent is selected from a library of known agents.

30. The method of claim 29, wherein the known agents are nonprotein agents.

31. A method of inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells, in a subject, comprising administering to the subject an agent capable of inhibiting interaction between CD40 ligand and CD40 on the cells, the agent being present in an amount effective to inhibit activation of the cells in the subject.

32. The method of claim 31, wherein the smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, aortic smooth muscle cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells.

33. The method of claim 32, wherein the gastrointestinal smooth muscle cells are esophageal smooth muscle cells, stomachic smooth muscle cells, smooth muscle cells of the small intestine, or smooth muscle cells of the large intestine.

34. The method of claim 31, wherein the agent inhibits binding of CD40 ligand to CD40 on the cells.

35. The method of claim 31, wherein the agent is a protein.

36. The method of claim 35, wherein the protein comprises an antibody or portion thereof.

37. The method of claim 36, wherein the antibody is a monoclonal antibody.

38. The method of claim 37, wherein the monoclonal antibody specifically binds to the epitope to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds.

39. The method of claim 38, wherein the agent is monoclonal antibody 5c8 (ATCC Accession No. HB 10916).

40. The method of claim 37, wherein the monoclonal antibody specifically binds to CD40.

41. The method of claim 40, wherein the antibody is humanized, chimeric, or primatized.

42. The method of claim 37, wherein the monoclonal antibody is a chimeric antibody.

43. The method of claim 37, wherein the monoclonal antibody is a humanized antibody.

44. The method of claim 36, wherein the portion of the antibody comprises a complementarity determining region or variable region of a light or heavy chain.

45. The method of claim 36, wherein the portion of the antibody comprises a complementarity determining region or a variable region.

46. The method of claim 45, wherein the portion of the antibody comprises a Fab or a single chain antibody.

47. The method of claim 31, wherein the subject is a mammal.

48. The method of claim 47, wherein the mammal is a rodent.

49. The method of claim 47, wherein the mammal is a human.

50. The method of claim 31, wherein the protein comprises soluble extracellular region of CD40 ligand, or variant thereof including conservative substituents, or portion thereof; or soluble extracellular region of CD40, or variant thereof including conservative substituents, or portion thereof.

51. The method of claim 50, wherein the soluble extracellular region of CD40 ligand or CD40 is a monomer.

52. The method of claim 50, wherein the soluble extracellular region of CD40 is an oligomer.

53. The method of claim 50, wherein the protein comprising soluble extracellular region of CD40 or portion thereof or CD40 ligand or portion thereof further comprises an Fc region fused to the extracellular region of CD40 or portion thereof or CD40 ligand or portion thereof.

54. The method of claim 53, wherein the Fc region is capable of binding to protein A or protein G.

55. The method of claim 53, wherein the Fc region comprises IgG, IgA, IgM, IgD, or IgE, or subclasses thereof.

56. The method of claim 55, wherein:

the IgG is IgG_1, IgG_2, IgG_3, or IgG_4; or

the IgA is IgA_1 or IgA_2.

57. The method of claim 31, wherein the agent is nonprotein.

58. The method of claim 57, wherein the agent is a small molecule.

59. The method of claim 31, wherein the agent is selected from a library of known agents.

60. The method of claim 31, wherein the agent is modified from a known agent.

61. The method of claim 60, wherein the modified agent is designed by structure optimization of a lead inhibitor based on a three-dimensional structure of a complex of soluble extracellular region of CD40 ligand or portion thereof with the lead inhibitor.

62. The method of claim 31, wherein the agent is selected by a screening method, which comprises:

isolating a sample of cells;

culturing the sample under conditions permitting activation of CD40-bearing cells;

contacting the sample with cells expressing a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with a protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, effective to activate the CD40-bearing cells;

contacting the sample with an amount of the agent effective to inhibit activation of the CD40-bearing cells if the agent is capable of inhibiting activation of the CD40-bearing cells; and

determining whether the cells expressing the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916, or with the protein which is specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 10916,activate the CD40-bearing cells in the presence of the agent.

63. The method of claim 62, wherein the agent is selected from a library of known agents.

64. The method of claim 63, wherein the known agents are nonprotein agents.

65. A method of treating, in a subject, a smooth muscle cell-dependent disease, comprising inhibiting activation by CD40 ligand of smooth muscle cells bearing CD40 on the surface of the cells according to the method of claim 31.

66. The method of claim 65, wherein the smooth muscle cell-dependent disease is a vascular disease.

67. The method of claim 66, wherein the vascular disease is atherosclerosis.

68. The method of claim 65, wherein the smooth muscle cell-dependent disease is a gastrointestinal disease.

69. The method of claim 68, wherein the gastrointestinal

disease is selected from the group consisting of: esophageal dysmotility, inflammatory bowel disease, and scleroderma.

70. The method of claim 65, wherein the smooth muscle cell-dependent disease is a bladder disease.

FIGURE 1A

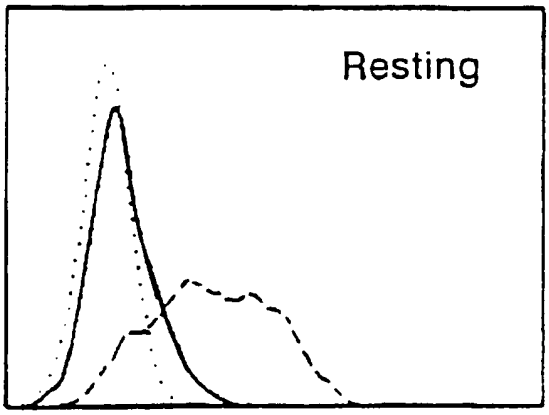

FIGURE 1B

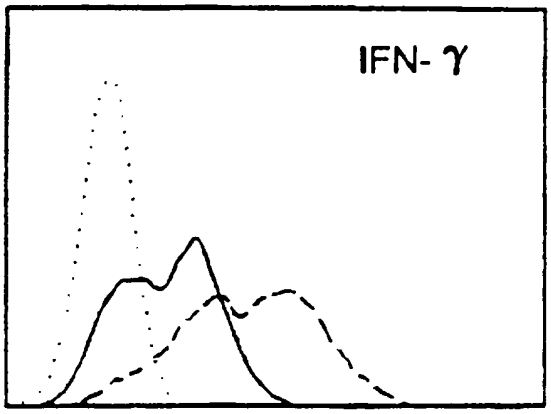

FIGURE 1C

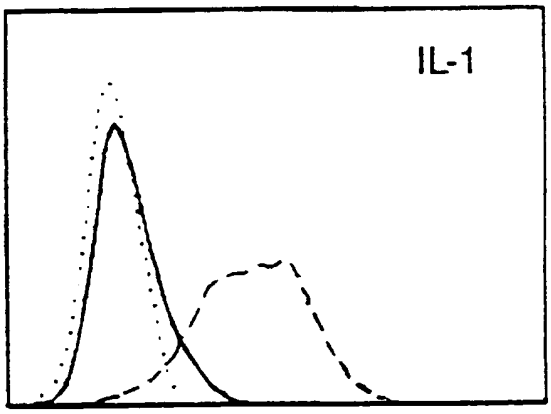

FIGURE 1D

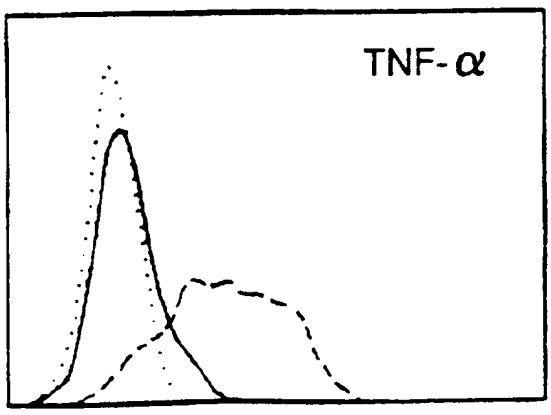

FIGURE 2A

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REMARKS ATOMIC COORDINATES OF CD40L CRYSTAL STRUCTURE IN PDB FORMAT
CRYST    77.170   77.170   90.460  90.00  90.00 120.00 R3
ATOM      1   N   GLY   116      -7.954 -16.144  22.488  1.00 64.71      A
ATOM      2   HT1 GLY   116      -7.087 -15.852  21.964  1.00 15.00      A
ATOM      3   HT2 GLY   116      -8.082 -17.142  22.242  1.00 15.00      A
ATOM      4   HT3 GLY   116      -8.630 -15.576  21.928  1.00 15.00      A
ATOM      5   CA  GLY   116      -7.927 -15.755  23.928  1.00 64.37      A
ATOM      6   C   GLY   116      -6.990 -16.621  24.780  1.00 64.34      A
ATOM      7   O   GLY   116      -6.968 -17.814  24.563  1.00 64.44      A
ATOM      8   N   ASP   117      -6.238 -16.043  25.740  1.00 64.04      A
ATOM      9   H   ASP   117      -5.617 -16.709  26.170  1.00 15.00      A
ATOM     10   CA  ASP   117      -6.284 -14.616  26.130  1.00 63.57      A
ATOM     11   CB  ASP   117      -5.711 -14.402  27.539  1.00 63.36      A
ATOM     12   CG  ASP   117      -6.518 -15.163  28.574  1.00 63.71      A
ATOM     13   OD1 ASP   117      -6.090 -16.247  28.965  1.00 63.24      A
ATOM     14   OD2 ASP   117      -7.566 -14.668  28.987  1.00 63.29      A
ATOM     15   C   ASP   117      -5.651 -13.585  25.184  1.00 63.31      A
ATOM     16   O   ASP   117      -6.039 -12.427  25.145  1.00 63.35      A
ATOM     17   N   GLN   118      -4.713 -14.090  24.379  1.00 62.72      A
ATOM     18   H   GLN   118      -4.450 -15.040  24.541  1.00 15.00      A
ATOM     19   CA  GLN   118      -4.097 -13.313  23.281  1.00 61.79      A
ATOM     20   CB  GLN   118      -2.918 -14.117  22.687  1.00 62.46      A
ATOM     21   CG  GLN   118      -3.047 -15.659  22.562  1.00 62.95      A
ATOM     22   CD  GLN   118      -4.277 -16.118  21.790  1.00 63.26      A
ATOM     23   OE1 GLN   118      -5.396 -16.000  22.277  1.00 63.43      A
ATOM     24   NE2 GLN   118      -4.044 -16.665  20.601  1.00 63.42      A
ATOM     25   HE21 GLN  118      -4.836 -16.715  19.975  1.00 15.00      A
ATOM     26   HE22 GLN  118      -3.151 -16.995  20.298  1.00 15.00      A
ATOM     27   C   GLN   118      -4.999 -12.841  22.128  1.00 60.59      A
ATOM     28   O   GLN   118      -4.887 -13.379  21.052  1.00 60.79      A
ATOM     29   N   ASN   119      -5.912 -11.901  22.445  1.00 58.61      A
ATOM     30   H   ASN   119      -5.917 -11.600  23.389  1.00 15.00      A
ATOM     31   CA  ASN   119      -6.689 -11.222  21.386  1.00 56.39      A
ATOM     32   CB  ASN   119      -7.947 -11.982  20.936  1.00 56.95      A
ATOM     33   CG  ASN   119      -7.652 -13.352  20.375  1.00 57.45      A
ATOM     34   OD1 ASN   119      -7.941 -14.303  21.084  1.00 58.50      A
ATOM     35   ND2 ASN   119      -7.005 -13.431  19.241  1.00 58.58      A
ATOM     36   HD21 ASN  119      -6.843 -12.617  18.646  1.00 15.00      A
ATOM     37   HD22 ASN  119      -6.740 -14.221  18.684  1.00 15.00      A
ATOM     38   C   ASN   119      -7.053  -9.724  21.571  1.00 53.62      A
ATOM     39   O   ASN   119      -6.746  -8.933  20.694  1.00 56.55      A
ATOM     40   N   PRO   120      -7.737  -9.288  22.698  1.00 50.17      A
ATOM     41   CD  PRO   120      -8.151 -10.129  23.810  1.00 51.90      A
ATOM     42   CA  PRO   120      -8.402  -7.945  22.818  1.00 48.19      A
ATOM     43   CB  PRO   120      -9.191  -8.008  24.117  1.00 47.42      A
ATOM     44   CG  PRO   120      -9.444  -9.493  24.321  1.00 51.93      A
ATOM     45   C   PRO   120      -7.750  -6.524  22.657  1.00 45.59      A
ATOM     46   O   PRO   120      -8.187  -5.516  23.225  1.00 45.37      A
ATOM     47   N   GLN   121      -6.789  -6.458  21.721  1.00 38.52      A
ATOM     48   H   GLN   121      -6.287  -7.304  21.505  1.00 15.00      A
ATOM     49   CA  GLN   121      -6.733  -5.359  20.753  1.00 29.14      A
ATOM     50   CB  GLN   121      -5.454  -5.735  19.971  1.00 26.30      A
ATOM     51   CG  GLN   121      -5.128  -4.943  18.710  1.00 26.84      A
ATOM     52   CD  GLN   121      -4.923  -3.460  18.949  1.00 27.26      A
ATOM     53   OE1 GLN   121      -5.822  -2.668  18.709  1.00 28.66      A
ATOM     54   NE2 GLN   121      -3.717  -3.100  19.341  1.00 33.90      A
ATOM     55   HE21 GLN  121       2.883  -3.614  19.564  1.00 15.00      A
ATOM     56   HE22 GLN  121      -3.442  -2.138  19.204  1.00 15.00      A
ATOM     57   C   GLN   121      -8.065  -5.218  19.903  1.00 26.33      A
ATOM     58   O   GLN   121      -8.905  -6.097  19.834  1.00 21.41      A
ATOM     59   N   ILE   122      -8.288  -4.051  19.272  1.00 21.21      A
```

FIGURE 2B

ATOM	60	H	ILE	122	-7.600	-3.320	19.337	1.00	15.00	A
ATOM	61	CA	ILE	122	-9.383	-3.952	18.295	1.00	20.92	A
ATOM	62	CB	ILE	122	-10.238	-2.629	18.396	1.00	22.17	A
ATOM	63	CG2	ILE	122	-11.275	-2.428	17.272	1.00	21.61	A
ATOM	64	CG1	ILE	122	-11.076	-2.744	19.668	1.00	24.13	A
ATOM	65	CD1	ILE	122	-11.751	-1.440	20.073	1.00	23.04	A
ATOM	66	C	ILE	122	-8.833	-4.108	16.895	1.00	18.96	A
ATOM	67	O	ILE	122	-8.135	-3.243	16.379	1.00	17.93	A
ATOM	68	N	ALA	123	-9.159	-5.240	16.283	1.00	14.72	A
ATOM	69	H	ALA	123	-9.599	-5.978	16.805	1.00	15.00	A
ATOM	70	CA	ALA	123	-8.656	-5.401	14.917	1.00	14.29	A
ATOM	71	CB	ALA	123	-7.176	-5.868	14.903	1.00	12.83	A
ATOM	72	C	ALA	123	-9.483	-6.315	13.985	1.00	15.66	A
ATOM	73	O	ALA	123	-10.170	-7.261	14.323	1.00	13.58	A
ATOM	74	N	ALA	124	-9.388	-6.009	12.724	1.00	13.45	A
ATOM	75	H	ALA	124	-8.894	-5.185	12.456	1.00	15.00	A
ATOM	76	CA	ALA	124	-10.087	-6.920	11.836	1.00	14.55	A
ATOM	77	CB	ALA	124	-11.486	-6.368	11.446	1.00	11.37	A
ATOM	78	C	ALA	124	-9.271	-7.123	10.563	1.00	13.54	A
ATOM	79	O	ALA	124	-8.501	-6.274	10.129	1.00	16.29	A
ATOM	80	N	HIS	125	-9.544	-8.248	9.937	1.00	11.49	A
ATOM	81	H	HIS	125	-10.100	-8.900	10.426	1.00	15.00	A
ATOM	82	CA	HIS	125	-9.100	-8.524	8.590	1.00	11.51	A
ATOM	83	CB	HIS	125	-7.605	-8.908	8.614	1.00	11.43	A
ATOM	84	CG	HIS	125	-7.119	-9.116	7.205	1.00	7.41	A
ATOM	85	ND1	HIS	125	-6.750	-8.130	6.421	1.00	6.60	A
ATOM	86	HD1	HIS	125	-6.708	-7.168	6.621	1.00	15.00	A
ATOM	87	CD2	HIS	125	-7.075	-10.291	6.456	1.00	12.36	A
ATOM	88	NE2	HIS	125	-6.670	-9.971	5.234	1.00	6.20	A
ATOM	89	CE1	HIS	125	-6.462	-8.646	5.211	1.00	4.48	A
ATOM	90	C	HIS	125	-10.024	-9.570	7.931	1.00	12.63	A
ATOM	91	O	HIS	125	-10.324	-10.650	8.383	1.00	13.14	A
ATOM	92	N	VAL	126	-10.550	-9.129	6.806	1.00	15.65	A
ATOM	93	H	VAL	126	-10.169	-8.286	6.428	1.00	15.00	A
ATOM	94	CA	VAL	126	-11.743	-9.717	6.201	1.00	14.38	A
ATOM	95	CB	VAL	126	-12.877	-8.808	6.675	1.00	13.37	A
ATOM	96	CG1	VAL	126	-13.794	-9.722	7.379	1.00	12.60	A
ATOM	97	CG2	VAL	126	-13.449	-7.663	5.814	1.00	9.61	A
ATOM	98	C	VAL	126	-11.502	-9.971	4.685	1.00	16.03	A
ATOM	99	O	VAL	126	-10.684	-9.297	4.074	1.00	16.42	A
ATOM	100	N	ILE	127	-12.118	-11.013	4.136	1.00	15.99	A
ATOM	101	H	ILE	127	-12.807	-11.481	4.691	1.00	15.00	A
ATOM	102	CA	ILE	127	-11.651	-11.532	2.831	1.00	14.86	A
ATOM	103	CB	ILE	127	-11.414	-13.051	3.002	1.00	17.56	A
ATOM	104	CG2	ILE	127	-11.716	-13.910	1.765	1.00	17.17	A
ATOM	105	CG1	ILE	127	-9.972	-13.316	3.399	1.00	16.47	A
ATOM	106	CD1	ILE	127	-9.705	-12.992	4.864	1.00	19.64	A
ATOM	107	C	ILE	127	-12.691	-11.269	1.765	1.00	18.96	A
ATOM	108	O	ILE	127	-13.898	-11.391	2.016	1.00	20.01	A
ATOM	109	N	SER	128	-12.229	-10.882	0.581	1.00	17.54	A
ATOM	110	H	SER	128	-11.232	-10.871	0.382	1.00	15.00	A
ATOM	111	CA	SER	128	-13.274	-10.667	-0.437	1.00	15.55	A
ATOM	112	CB	SER	128	-12.664	-10.130	-1.706	1.00	18.16	A
ATOM	113	OG	SER	128	-12.205	-11.207	-2.574	1.00	19.90	A
ATOM	114	HG	SER	128	-11.832	-11.931	-2.029	1.00	15.00	A
ATOM	115	C	SER	128	-14.295	-11.761	-0.792	1.00	13.62	A
ATOM	116	O	SER	128	-14.052	-12.960	-0.832	1.00	8.98	A
ATOM	117	N	GLU	129	-15.492	-11.246	-1.027	1.00	13.36	A
ATOM	118	H	GLU	129	-15.661	-10.257	-0.937	1.00	15.00	A
ATOM	119	CA	GLU	129	-16.379	-12.024	-1.840	1.00	17.20	A

FIGURE 2C

ATOM	120	CB	GLU	129	-17.052	-13.117	-1.021	1.00	20.55	A
ATOM	121	CG	GLU	129	-18.092	-12.694	-0.036	1.00	17.92	A
ATOM	122	CD	GLU	129	-18.781	-13.951	0.376	1.00	21.98	A
ATOM	123	OE1	GLU	129	-19.997	-13.932	0.368	1.00	32.23	A
ATOM	124	OE2	GLU	129	-18.150	-14.938	0.734	1.00	33.12	A
ATOM	125	C	GLU	129	-17.371	-11.409	-2.809	1.00	17.71	A
ATOM	126	O	GLU	129	-17.972	-10.389	-2.553	1.00	21.59	A
ATOM	127	N	ALA	130	-17.550	-12.145	-3.914	1.00	20.52	A
ATOM	128	H	ALA	130	-17.136	-13.057	-3.923	1.00	15.00	A
ATOM	129	CA	ALA	130	-18.379	-11.649	-5.019	1.00	23.36	A
ATOM	130	CB	ALA	130	-18.424	-12.633	-6.208	1.00	19.66	A
ATOM	131	C	ALA	130	-19.811	-11.298	-4.570	1.00	26.86	A
ATOM	132	O	ALA	130	-20.519	-12.022	-3.869	1.00	29.40	A
ATOM	133	N	SER	131	-20.198	-10.086	-4.968	1.00	21.70	A
ATOM	134	H	SER	131	-19.515	-9.481	-5.410	1.00	15.00	A
ATOM	135	CA	SER	131	-21.592	-9.782	-4.732	1.00	20.04	A
ATOM	136	CB	SER	131	-21.829	-8.266	-4.787	1.00	20.65	A
ATOM	137	OG	SER	131	-23.182	-8.001	-4.435	1.00	15.24	A
ATOM	138	HG	SER	131	-23.329	-7.069	-4.559	1.00	15.00	A
ATOM	139	C	SER	131	-22.546	-10.501	-5.668	1.00	17.15	A
ATOM	140	O	SER	131	-22.236	-10.853	-6.786	1.00	14.30	A
ATOM	141	N	SER	132	-23.756	-10.731	-5.187	1.00	20.15	A
ATOM	142	H	SER	132	-23.967	-10.586	-4.209	1.00	15.00	A
ATOM	143	CA	SER	132	-24.674	-11.250	-6.218	1.00	21.62	A
ATOM	144	CB	SER	132	-25.266	-12.616	-5.893	1.00	16.00	A
ATOM	145	OG	SER	132	-26.203	-12.324	-4.894	1.00	23.84	A
ATOM	146	HG	SER	132	-26.016	-12.944	-4.179	1.00	15.00	A
ATOM	147	C	SER	132	-25.727	-10.268	-6.671	1.00	20.07	A
ATOM	148	O	SER	132	-26.535	-10.544	-7.547	1.00	20.27	A
ATOM	149	N	LYS	133	-25.606	-9.063	-6.118	1.00	21.87	A
ATOM	150	H	LYS	133	-24.904	-8.969	-5.397	1.00	15.00	A
ATOM	151	CA	LYS	133	-26.406	-7.916	-6.517	1.00	19.23	A
ATOM	152	CB	LYS	133	-27.024	-7.309	-5.256	1.00	23.08	A
ATOM	153	CG	LYS	133	-27.684	-8.364	-4.354	1.00	21.07	A
ATOM	154	CD	LYS	133	-29.174	-8.110	-4.330	1.00	27.36	A
ATOM	155	CE	LYS	133	-29.939	-7.884	-5.670	1.00	30.56	A
ATOM	156	NZ	LYS	133	-31.323	-7.515	-5.345	1.00	21.56	A
ATOM	157	HZ1	LYS	133	-31.862	-7.351	-6.218	1.00	15.00	A
ATOM	158	HZ2	LYS	133	-31.753	-8.299	-4.811	1.00	15.00	A
ATOM	159	HZ3	LYS	133	-31.333	-6.654	-4.760	1.00	15.00	A
ATOM	160	C	LYS	133	-25.579	-6.876	-7.194	1.00	20.10	A
ATOM	161	O	LYS	133	-24.378	-6.801	-7.007	1.00	17.94	A
ATOM	162	N	THR	134	-26.260	-6.052	-7.983	1.00	22.95	A
ATOM	163	H	THR	134	-27.275	-6.130	-8.036	1.00	15.00	A
ATOM	164	CA	THR	134	-25.556	-4.879	-8.561	1.00	27.89	A
ATOM	165	CB	THR	134	-26.498	-4.274	-9.592	1.00	24.59	A
ATOM	166	OG1	THR	134	-26.540	-5.037	-10.792	1.00	24.32	A
ATOM	167	HG1	THR	134	-26.232	-4.411	-11.456	1.00	15.00	A
ATOM	168	CG2	THR	134	-26.044	-2.897	-9.968	1.00	22.97	A
ATOM	169	C	THR	134	-24.987	-3.798	-7.559	1.00	32.51	A
ATOM	170	O	THR	134	-25.658	-3.461	-6.603	1.00	38.43	A
ATOM	171	N	THR	135	-23.717	-3.352	-7.690	1.00	35.98	A
ATOM	172	H	THR	135	-23.292	-3.555	-8.585	1.00	15.00	A
ATOM	173	CA	THR	135	-22.964	-3.469	-6.386	1.00	36.02	A
ATOM	174	CB	THR	135	-21.575	-4.276	-6.534	1.00	36.01	A
ATOM	175	OG1	THR	135	-21.645	-5.388	-7.488	1.00	30.60	A
ATOM	176	HG1	THR	135	-22.255	-6.094	-7.312	1.00	15.00	A
ATOM	177	CG2	THR	135	-20.866	-4.776	-5.264	1.00	35.55	A
ATOM	178	C	THR	135	-22.949	-2.266	-5.404	1.00	30.25	A
ATOM	179	C	THR	135	-23.541	-2.348	-4.331	1.00	28.35	A

FIGURE 2D

ATOM	180	N	SER	136	-22.294	-1.146	-5.776	1.00	23.29	A
ATOM	181	H	SER	136	-22.828	-0.357	-5.460	1.00	15.00	A
ATOM	182	CA	SER	136	-20.857	-1.051	-6.143	1.00	23.04	A
ATOM	183	CB	SER	136	-20.560	0.187	-6.965	1.00	21.03	A
ATOM	184	OG	SER	136	-20.624	1.261	-6.043	1.00	28.21	A
ATOM	185	HG	SER	136	-19.815	1.793	-6.008	1.00	15.00	A
ATOM	186	C	SER	136	-19.853	-1.090	-4.958	1.00	21.77	A
ATOM	187	O	SER	136	-18.630	-1.096	-5.080	1.00	21.94	A
ATOM	188	N	VAL	137	-20.452	-1.227	-3.752	1.00	24.03	A
ATOM	189	H	VAL	137	-21.440	-1.063	-3.705	1.00	15.00	A
ATOM	190	CA	VAL	137	-19.699	-1.632	-2.570	1.00	19.65	A
ATOM	191	CB	VAL	137	-20.218	-1.010	-1.248	1.00	21.14	A
ATOM	192	CG1	VAL	137	-20.419	-1.907	-0.058	1.00	18.16	A
ATOM	193	CG2	VAL	137	-21.322	-0.026	-1.442	1.00	13.49	A
ATOM	194	C	VAL	137	-19.370	-3.116	-2.473	1.00	17.15	A
ATOM	195	O	VAL	137	-20.209	-3.969	-2.593	1.00	16.69	A
ATOM	196	N	LEU	138	-18.077	-3.344	-2.271	1.00	15.84	A
ATOM	197	H	LEU	138	-17.502	-2.528	-2.246	1.00	15.00	A
ATOM	198	CA	LEU	138	-17.507	-4.667	-1.938	1.00	18.21	A
ATOM	199	CB	LEU	138	-15.962	-4.530	-1.791	1.00	13.60	A
ATOM	200	CG	LEU	138	-15.273	-3.854	-2.998	1.00	16.09	A
ATOM	201	CD1	LEU	138	-15.923	-4.379	-4.300	1.00	20.35	A
ATOM	202	CD2	LEU	138	-13.710	-3.936	-2.982	1.00	12.34	A
ATOM	203	C	LEU	138	-18.170	-5.480	-0.772	1.00	16.29	A
ATOM	204	O	LEU	138	-18.498	-4.986	0.301	1.00	12.97	A
ATOM	205	N	GLN	139	-18.345	-6.768	-1.035	1.00	13.04	A
ATOM	206	H	GLN	139	-18.052	-7.078	-1.960	1.00	15.00	A
ATOM	207	CA	GLN	139	-18.757	-7.658	0.013	1.00	15.32	A
ATOM	208	CB	GLN	139	-19.847	-8.678	-0.481	1.00	13.99	A
ATOM	209	CG	GLN	139	-21.068	-7.960	-1.113	1.00	20.85	A
ATOM	210	CD	GLN	139	-21.872	-7.022	-0.193	1.00	22.04	A
ATOM	211	OE1	GLN	139	-22.343	-7.439	0.878	1.00	25.45	A
ATOM	212	NE2	GLN	139	-21.963	-5.739	-0.618	1.00	17.74	A
ATOM	213	HE21	GLN	139	-22.697	-5.181	-0.206	1.00	15.00	A
ATOM	214	HE22	GLN	139	-21.460	-5.326	-1.374	1.00	15.00	A
ATOM	215	C	GLN	139	-17.527	-8.383	0.541	1.00	14.26	A
ATOM	216	O	GLN	139	-16.554	-8.640	-0.144	1.00	14.40	A
ATOM	217	N	TRP	140	-17.647	-8.780	1.805	1.00	12.80	A
ATOM	218	H	TRP	140	-18.433	-8.447	2.297	1.00	15.00	A
ATOM	219	CA	TRP	140	-16.542	-9.500	2.463	1.00	14.03	A
ATOM	220	CB	TRP	140	-15.813	-8.623	3.483	1.00	14.18	A
ATOM	221	CG	TRP	140	-15.467	-7.291	2.823	1.00	8.44	A
ATOM	222	CD2	TRP	140	-14.379	-6.966	1.941	1.00	9.01	A
ATOM	223	CE2	TRP	140	-14.549	-5.625	1.482	1.00	8.40	A
ATOM	224	CE3	TRP	140	-13.215	-7.688	1.581	1.00	10.14	A
ATOM	225	CD1	TRP	140	-16.225	-6.137	2.863	1.00	11.29	A
ATOM	226	NE1	TRP	140	-15.710	-5.150	2.077	1.00	14.27	A
ATOM	227	HE1	TRP	140	-16.121	-4.268	2.010	1.00	15.00	A
ATOM	228	CZ2	TRP	140	-13.640	-5.009	0.590	1.00	8.16	A
ATOM	229	CZ3	TRP	140	-12.292	-7.069	0.713	1.00	13.90	A
ATOM	230	CH2	TRP	140	-12.497	-5.749	0.215	1.00	12.11	A
ATOM	231	C	TRP	140	-17.016	-10.701	3.170	1.00	14.34	A
ATOM	232	O	TRP	140	-18.193	-10.862	3.392	1.00	16.00	A
ATOM	233	N	ALA	141	-16.082	-11.528	3.558	1.00	14.80	A
ATOM	234	H	ALA	141	-15.133	-11.377	3.294	1.00	15.00	A
ATOM	235	CA	ALA	141	-16.489	-12.617	4.394	1.00	15.27	A
ATOM	236	CB	ALA	141	-16.504	-13.920	3.583	1.00	16.97	A
ATOM	237	C	ALA	141	-15.585	-12.761	5.607	1.00	15.90	A
ATOM	238	O	ALA	141	-14.453	-12.338	5.550	1.00	14.25	A
ATOM	239	N	GLU	142	-16.068	-13.366	6.688	1.00	19.74	A

FIGURE 2E

ATOM	240	H	GLU	142	-17.055	-13.574	6.688	1.00	15.00	A
ATOM	241	CA	GLU	142	-15.149	-13.759	7.731	1.00	25.93	A
ATOM	242	CB	GLU	142	-15.794	-13.910	9.117	1.00	21.75	A
ATOM	243	CG	GLU	142	-15.716	-12.456	9.647	1.00	24.05	A
ATOM	244	CD	GLU	142	-16.749	-12.087	10.711	1.00	26.61	A
ATOM	245	OE1	GLU	142	-17.908	-11.888	10.361	1.00	34.72	A
ATOM	246	OE2	GLU	142	-16.404	-11.984	11.886	1.00	30.07	A
ATOM	247	C	GLU	142	-14.200	-14.797	7.193	1.00	33.25	A
ATOM	248	O	GLU	142	-13.156	-14.349	6.737	1.00	41.84	A
ATOM	249	N	LYS	143	-14.577	-16.080	7.084	1.00	34.17	A
ATOM	250	H	LYS	143	-15.432	-16.384	7.492	1.00	15.00	A
ATOM	251	CA	LYS	143	-13.882	-16.854	5.980	1.00	35.31	A
ATOM	252	CB	LYS	143	-14.673	-16.603	4.681	1.00	37.64	A
ATOM	253	CG	LYS	143	-14.300	-17.505	3.531	1.00	47.37	A
ATOM	254	CD	LYS	143	-15.022	-17.284	2.202	1.00	50.37	A
ATOM	255	CE	LYS	143	-14.686	-16.047	1.357	1.00	49.23	A
ATOM	256	NZ	LYS	143	-15.632	-16.097	0.221	1.00	51.67	A
ATOM	257	HZ1	LYS	143	-15.333	-15.445	-0.534	1.00	15.00	A
ATOM	258	HZ2	LYS	143	-15.680	-17.061	-0.177	1.00	15.00	A
ATOM	259	HZ3	LYS	143	-16.564	-15.833	0.585	1.00	15.00	A
ATOM	260	C	LYS	143	-12.330	-16.979	5.637	1.00	32.80	A
ATOM	261	O	LYS	143	-11.831	-18.041	5.276	1.00	35.64	A
ATOM	262	N	GLY	144	-11.522	-15.923	5.637	1.00	28.26	A
ATOM	263	H	GLY	144	-11.718	-14.995	5.910	1.00	15.00	A
ATOM	264	CA	GLY	144	-10.243	-16.458	5.194	1.00	32.94	A
ATOM	265	C	GLY	144	-9.178	-16.862	6.180	1.00	29.93	A
ATOM	266	O	GLY	144	-9.345	-17.454	7.205	1.00	24.67	A
ATOM	267	N	TYR	145	-8.069	-16.270	5.815	1.00	26.37	A
ATOM	268	H	TYR	145	-8.160	-15.729	4.966	1.00	15.00	A
ATOM	269	CA	TYR	145	-7.027	-16.002	6.777	1.00	27.61	A
ATOM	270	CB	TYR	145	-5.708	-15.877	5.947	1.00	37.54	A
ATOM	271	CG	TYR	145	-5.962	-15.774	4.456	1.00	50.95	A
ATOM	272	CD1	TYR	145	-5.682	-14.633	3.706	1.00	53.22	A
ATOM	273	CE1	TYR	145	-6.313	-14.377	2.468	1.00	60.28	A
ATOM	274	CD2	TYR	145	-6.591	-16.847	3.791	1.00	53.11	A
ATOM	275	CE2	TYR	145	-7.207	-16.699	2.551	1.00	56.30	A
ATOM	276	CZ	TYR	145	-7.162	-15.430	1.873	1.00	61.12	A
ATOM	277	OH	TYR	145	-7.812	-15.119	0.665	1.00	62.63	A
ATOM	278	HH	TYR	145	-8.575	-15.686	0.401	1.00	15.00	A
ATOM	279	C	TYR	145	-7.532	-14.762	7.620	1.00	22.41	A
ATOM	280	O	TYR	145	-7.000	-13.677	7.650	1.00	22.68	A
ATOM	281	N	TYR	146	-8.731	-14.884	8.196	1.00	20.39	A
ATOM	282	H	TYR	146	-8.935	-15.824	8.509	1.00	15.00	A
ATOM	283	CA	TYR	146	-9.423	-13.700	8.725	1.00	20.40	A
ATOM	284	CB	TYR	146	-10.886	-13.673	8.306	1.00	22.53	A
ATOM	285	CG	TYR	146	-11.710	-14.460	9.286	1.00	23.02	A
ATOM	286	CD1	TYR	146	-11.635	-15.873	9.236	1.00	26.99	A
ATOM	287	CE1	TYR	146	-12.254	-16.623	10.239	1.00	25.44	A
ATOM	288	CD2	TYR	146	-12.477	-13.766	10.236	1.00	23.45	A
ATOM	289	CE2	TYR	146	-13.150	-14.520	11.205	1.00	26.81	A
ATOM	290	CZ	TYR	146	-13.007	-15.937	11.204	1.00	27.40	A
ATOM	291	OH	TYR	146	-13.647	-16.689	12.170	1.00	31.91	A
ATOM	292	HH	TYR	146	-12.911	-17.080	12.676	1.00	15.00	A
ATOM	293	C	TYR	146	-9.291	-13.419	10.219	1.00	18.79	A
ATOM	294	O	TYR	146	-8.904	-14.232	11.012	1.00	16.13	A
ATOM	295	N	THR	147	-9.596	-12.169	10.556	1.00	17.54	A
ATOM	296	H	THR	147	-9.973	-11.607	9.830	1.00	15.00	A
ATOM	297	CA	THR	147	-9.432	-11.764	11.948	1.00	14.06	A
ATOM	298	CB	THR	147	-8.162	-10.875	12.182	1.00	13.66	A
ATOM	299	OG1	THR	147	-6.912	-11.505	11.856	1.00	12.56	A

FIGURE 2F

ATOM	300	HG1	THR	147	-6.934	-11.898	10.980	1.00	15.00	A
ATOM	301	CG2	THR	147	-8.025	-10.236	13.554	1.00	7.22	A
ATOM	302	C	THR	147	-10.619	-10.925	12.253	1.00	15.60	A
ATOM	303	O	THR	147	-11.044	-10.074	11.496	1.00	16.39	A
ATOM	304	N	MET	148	-11.144	-11.139	13.412	1.00	20.67	A
ATOM	305	H	MET	148	-10.838	-11.988	13.828	1.00	15.00	A
ATOM	306	CA	MET	148	-12.124	-10.311	14.110	1.00	19.71	A
ATOM	307	CB	MET	148	-13.546	-10.702	13.705	1.00	17.89	A
ATOM	308	CG	MET	148	-14.541	-9.580	14.019	1.00	13.53	A
ATOM	309	SD	MET	148	-14.492	-8.149	12.952	1.00	14.69	A
ATOM	310	CE	MET	148	-14.566	-8.928	11.333	1.00	10.10	A
ATOM	311	C	MET	148	-11.915	-10.282	15.639	1.00	21.49	A
ATOM	312	O	MET	148	-12.594	-10.905	16.436	1.00	22.98	A
ATOM	313	N	SER	149	-10.955	-9.412	16.055	1.00	20.58	A
ATOM	314	H	SER	149	-10.516	-8.786	15.406	1.00	15.00	A
ATOM	315	CA	SER	149	-10.388	-9.698	17.419	1.00	19.11	A
ATOM	316	CB	SER	149	-9.174	-8.860	17.792	1.00	12.17	A
ATOM	317	OG	SER	149	-9.540	-7.513	17.975	1.00	14.10	A
ATOM	318	HG	SER	149	-9.571	-7.487	18.934	1.00	15.00	A
ATOM	319	C	SER	149	-11.203	-9.844	18.727	1.00	22.19	A
ATOM	320	O	SER	149	-10.728	-10.267	19.772	1.00	22.95	A
ATOM	321	N	ASN	150	-12.456	-9.322	18.631	1.00	22.71	A
ATOM	322	H	ASN	150	-12.782	-9.247	17.688	1.00	15.00	A
ATOM	323	CA	ASN	150	-13.361	-9.236	19.764	1.00	20.32	A
ATOM	324	CB	ASN	150	-12.734	-8.446	20.955	1.00	21.56	A
ATOM	325	CG	ASN	150	-12.343	-6.962	20.706	1.00	20.71	A
ATOM	326	OD1	ASN	150	-13.059	-6.187	20.119	1.00	17.81	A
ATOM	327	ND2	ASN	150	-11.222	-6.485	21.271	1.00	23.86	A
ATOM	328	HD21	ASN	150	-11.035	-5.521	21.092	1.00	15.00	A
ATOM	329	HD22	ASN	150	-10.670	-7.109	21.821	1.00	15.00	A
ATOM	330	C	ASN	150	-14.644	-8.657	19.256	1.00	20.60	A
ATOM	331	O	ASN	150	-14.718	-8.130	18.148	1.00	20.56	A
ATOM	332	N	ASN	151	-15.637	-8.713	20.149	1.00	23.49	A
ATOM	333	H	ASN	151	-15.455	-9.124	21.038	1.00	15.00	A
ATOM	334	CA	ASN	151	-16.974	-8.080	19.823	1.00	24.71	A
ATOM	335	CB	ASN	151	-18.130	-8.645	20.712	1.00	28.30	A
ATOM	336	CG	ASN	151	-17.959	-8.271	22.173	1.00	33.23	A
ATOM	337	OD1	ASN	151	-17.075	-7.562	22.606	1.00	39.79	A
ATOM	338	ND2	ASN	151	-18.782	-8.838	23.011	1.00	38.32	A
ATOM	339	HD21	ASN	151	-18.553	-8.524	23.928	1.00	15.00	A
ATOM	340	HD22	ASN	151	-19.495	-9.465	22.733	1.00	15.00	A
ATOM	341	C	ASN	151	-17.172	-6.531	19.645	1.00	22.53	A
ATOM	342	O	ASN	151	-18.254	-6.048	19.374	1.00	21.32	A
ATOM	343	N	LEU	152	-16.066	-5.762	19.859	1.00	23.00	A
ATOM	344	H	LEU	152	-15.247	-6.289	20.070	1.00	15.00	A
ATOM	345	CA	LEU	152	-15.924	-4.335	19.525	1.00	18.87	A
ATOM	346	CB	LEU	152	-14.830	-3.700	20.325	1.00	21.77	A
ATOM	347	CG	LEU	152	-14.981	-3.999	21.806	1.00	24.80	A
ATOM	348	CD1	LEU	152	-16.390	-3.645	22.316	1.00	22.82	A
ATOM	349	CD2	LEU	152	-13.847	-3.256	22.556	1.00	23.56	A
ATOM	350	C	LEU	152	-15.565	-3.993	18.094	1.00	17.34	A
ATOM	351	O	LEU	152	-15.590	-2.840	17.708	1.00	13.39	A
ATOM	352	N	VAL	153	-15.267	-5.054	17.309	1.00	18.65	A
ATOM	353	H	VAL	153	-15.156	-5.962	17.716	1.00	15.00	A
ATOM	354	CA	VAL	153	-15.439	-4.910	15.849	1.00	16.81	A
ATOM	355	CB	VAL	153	-14.138	-5.021	14.980	1.00	15.33	A
ATOM	356	CG1	VAL	153	-12.908	-5.718	15.562	1.00	21.22	A
ATOM	357	CG2	VAL	153	-13.775	-3.757	14.287	1.00	16.95	A
ATOM	358	C	VAL	153	-16.405	-5.964	15.301	1.00	13.48	A
ATOM	359	O	VAL	153	-16.363	-7.116	15.647	1.00	13.06	A

FIGURE 2G

ATOM	360	N	THR	154	-17.207	-5.546	14.358	1.00	12.06	A
ATOM	361	H	THR	154	-17.313	-4.568	14.215	1.00	15.00	A
ATOM	362	CA	THR	154	-17.903	-6.600	13.615	1.00	16.26	A
ATOM	363	CB	THR	154	-19.366	-6.747	14.157	1.00	19.51	A
ATOM	364	OG1	THR	154	-19.995	-5.459	14.205	1.00	19.31	A
ATOM	365	HG1	THR	154	-20.577	-5.508	14.949	1.00	15.00	A
ATOM	366	CG2	THR	154	-19.502	-7.288	15.571	1.00	21.62	A
ATOM	367	C	THR	154	-17.997	-6.252	12.107	1.00	18.12	A
ATOM	368	O	THR	154	-17.992	-5.110	11.605	1.00	16.55	A
ATOM	369	N	LEU	155	-18.101	-7.324	11.357	1.00	16.77	A
ATOM	370	H	LEU	155	-18.056	-8.202	11.791	1.00	15.00	A
ATOM	371	CA	LEU	155	-18.514	-7.198	9.967	1.00	17.10	A
ATOM	372	CB	LEU	155	-17.829	-8.353	9.204	1.00	20.04	A
ATOM	373	CG	LEU	155	-17.524	-8.428	7.692	1.00	20.81	A
ATOM	374	CD1	LEU	155	-17.822	-7.159	6.908	1.00	17.03	A
ATOM	375	CD2	LEU	155	-17.912	-9.810	7.139	1.00	12.42	A
ATOM	376	C	LEU	155	-20.055	-7.187	9.904	1.00	20.71	A
ATOM	377	O	LEU	155	-20.712	-8.163	10.217	1.00	18.01	A
ATOM	378	N	GLU	156	-20.593	-5.995	9.561	1.00	19.51	A
ATOM	379	H	GLU	156	-19.959	-5.230	9.440	1.00	15.00	A
ATOM	380	CA	GLU	156	-22.036	-5.888	9.413	1.00	21.95	A
ATOM	381	CB	GLU	156	-22.641	-4.631	10.033	1.00	18.95	A
ATOM	382	CG	GLU	156	-22.098	-4.412	11.436	1.00	27.68	A
ATOM	383	CD	GLU	156	-22.721	-5.194	12.587	1.00	31.62	A
ATOM	384	OE1	GLU	156	-23.347	-6.248	12.367	1.00	33.40	A
ATOM	385	OE2	GLU	156	-22.532	-4.721	13.724	1.00	35.00	A
ATOM	386	C	GLU	156	-22.457	-5.966	7.964	1.00	25.36	A
ATOM	387	O	GLU	156	-21.958	-5.298	7.077	1.00	22.70	A
ATOM	388	N	ASN	157	-23.437	-6.808	7.696	1.00	30.92	A
ATOM	389	H	ASN	157	-23.594	-7.590	8.300	1.00	15.00	A
ATOM	390	CA	ASN	157	-23.804	-6.620	6.300	1.00	33.31	A
ATOM	391	CB	ASN	157	-23.856	-7.970	5.614	1.00	31.69	A
ATOM	392	CG	ASN	157	-23.669	-7.693	4.168	1.00	27.70	A
ATOM	393	OD1	ASN	157	-23.397	-6.593	3.810	1.00	25.89	A
ATOM	394	ND2	ASN	157	-23.893	-8.640	3.275	1.00	41.69	A
ATOM	395	HD21	ASN	157	-24.069	-9.603	3.467	1.00	15.00	A
ATOM	396	HD22	ASN	157	-23.745	-8.295	2.340	1.00	15.00	A
ATOM	397	C	ASN	157	-24.988	-5.658	6.118	1.00	35.08	A
ATOM	398	O	ASN	157	-26.107	-5.949	6.499	1.00	37.06	A
ATOM	399	N	GLY	158	-24.746	-4.443	5.560	1.00	40.03	A
ATOM	400	H	GLY	158	-25.601	-3.952	5.429	1.00	15.00	A
ATOM	401	CA	GLY	158	-23.422	-3.887	5.121	1.00	38.11	A
ATOM	402	C	GLY	158	-23.062	-3.720	3.617	1.00	37.48	A
ATOM	403	O	GLY	158	-23.890	-3.108	2.950	1.00	41.11	A
ATOM	404	N	LYS	159	-21.867	-4.220	3.135	1.00	32.75	A
ATOM	405	H	LYS	159	-21.904	-4.134	2.130	1.00	15.00	A
ATOM	406	CA	LYS	159	-20.828	-4.928	3.962	1.00	27.83	A
ATOM	407	CB	LYS	159	-20.317	-6.122	3.217	1.00	28.17	A
ATOM	408	CG	LYS	159	-19.734	-7.168	4.069	1.00	20.48	A
ATOM	409	CD	LYS	159	-20.533	-8.426	4.192	1.00	29.61	A
ATOM	410	CE	LYS	159	-20.577	-9.191	2.869	1.00	40.41	A
ATOM	411	NZ	LYS	159	-20.796	-10.663	2.986	1.00	40.88	A
ATOM	412	HZ1	LYS	159	-20.739	-11.087	2.035	1.00	15.00	A
ATOM	413	HZ2	LYS	159	-20.070	-11.087	3.600	1.00	15.00	A
ATOM	414	HZ3	LYS	159	-21.738	-10.848	3.389	1.00	15.00	A
ATOM	415	C	LYS	159	-19.688	-4.065	4.463	1.00	26.08	A
ATOM	416	O	LYS	159	-19.023	-3.369	3.696	1.00	28.01	A
ATOM	417	N	GLN	160	-19.683	-3.990	5.807	1.00	18.90	A
ATOM	418	H	GLN	160	-20.211	-4.674	6.319	1.00	15.00	A
ATOM	419	CA	GLN	160	-18.922	-2.929	6.464	1.00	13.89	A

FIGURE 2H

ATOM	420	CB	GLN	160	-19.778	-1.694	6.611	1.00	16.79	A
ATOM	421	CG	GLN	160	-20.881	-1.896	7.633	1.00	18.34	A
ATOM	422	CD	GLN	160	-22.133	-1.166	7.193	1.00	23.97	A
ATOM	423	OE1	GLN	160	-23.088	-0.970	7.893	1.00	31.18	A
ATOM	424	NE2	GLN	160	-22.257	-0.771	5.948	1.00	28.16	A
ATOM	425	HE21	GLN	160	-23.194	-0.420	5.928	1.00	15.00	A
ATOM	426	HE22	GLN	160	-21.624	-0.780	5.186	1.00	15.00	A
ATOM	427	C	GLN	160	-18.313	-3.309	7.777	1.00	12.67	A
ATOM	428	O	GLN	160	-18.838	-4.151	8.498	1.00	14.78	A
ATOM	429	N	LEU	161	-17.187	-2.637	8.085	1.00	11.22	A
ATOM	430	H	LEU	161	-16.767	-2.124	7.340	1.00	15.00	A
ATOM	431	CA	LEU	161	-16.583	-2.870	9.405	1.00	9.71	A
ATOM	432	CB	LEU	161	-15.052	-2.939	9.390	1.00	4.67	A
ATOM	433	CG	LEU	161	-14.438	-4.060	8.559	1.00	7.30	A
ATOM	434	CD1	LEU	161	-14.511	-5.447	9.207	1.00	10.80	A
ATOM	435	CD2	LEU	161	-12.964	-3.794	8.389	1.00	5.48	A
ATOM	436	C	LEU	161	-17.082	-1.836	10.412	1.00	10.17	A
ATOM	437	O	LEU	161	-16.826	-0.657	10.341	1.00	13.36	A
ATOM	438	N	THR	162	-17.848	-2.338	11.375	1.00	16.94	A
ATOM	439	H	THR	162	-18.153	-3.279	11.251	1.00	15.00	A
ATOM	440	CA	THR	162	-18.317	-1.480	12.493	1.00	16.14	A
ATOM	441	CB	THR	162	-19.807	-1.769	12.640	1.00	13.33	A
ATOM	442	OG1	THR	162	-20.339	-1.707	11.308	1.00	16.73	A
ATOM	443	HG1	THR	162	-21.211	-1.254	11.343	1.00	15.00	A
ATOM	444	CG2	THR	162	-20.553	-0.832	13.562	1.00	15.01	A
ATOM	445	C	THR	162	-17.531	-1.547	13.842	1.00	13.28	A
ATOM	446	O	THR	162	-17.358	-2.587	14.449	1.00	20.21	A
ATOM	447	N	VAL	163	-16.994	-0.437	14.282	1.00	14.22	A
ATOM	448	H	VAL	163	-16.859	0.243	13.567	1.00	15.00	A
ATOM	449	CA	VAL	163	-16.326	-0.358	15.586	1.00	15.72	A
ATOM	450	CB	VAL	163	-15.038	0.426	15.428	1.00	11.82	A
ATOM	451	CG1	VAL	163	-15.191	1.944	15.368	1.00	9.87	A
ATOM	452	CG2	VAL	163	-14.229	-0.124	14.245	1.00	18.88	A
ATOM	453	C	VAL	163	-17.193	0.283	16.706	1.00	17.93	A
ATOM	454	O	VAL	163	-18.001	1.180	16.453	1.00	20.25	A
ATOM	455	N	LYS	164	-17.037	-0.232	17.925	1.00	15.44	A
ATOM	456	H	LYS	164	-16.254	-0.858	18.020	1.00	15.00	A
ATOM	457	CA	LYS	164	-17.856	0.138	19.109	1.00	17.33	A
ATOM	458	CB	LYS	164	-18.351	-1.150	19.807	1.00	19.58	A
ATOM	459	CG	LYS	164	-19.214	-1.885	18.759	1.00	23.56	A
ATOM	460	CD	LYS	164	-19.417	-3.410	18.851	1.00	28.85	A
ATOM	461	CE	LYS	164	-20.039	-4.047	17.554	1.00	33.81	A
ATOM	462	NZ	LYS	164	-19.428	-3.681	16.227	1.00	18.98	A
ATOM	463	HZ1	LYS	164	-19.195	-2.667	16.222	1.00	15.00	A
ATOM	464	HZ2	LYS	164	-18.552	-4.223	16.092	1.00	15.00	A
ATOM	465	HZ3	LYS	164	-20.084	-3.888	15.445	1.00	15.00	A
ATOM	466	C	LYS	164	-17.193	1.099	20.056	1.00	15.14	A
ATOM	467	O	LYS	164	-17.712	1.588	21.048	1.00	17.72	A
ATOM	468	N	ARG	165	-15.992	1.428	19.621	1.00	17.49	A
ATOM	469	H	ARG	165	-15.550	0.838	18.932	1.00	15.00	A
ATOM	470	CA	ARG	165	-15.184	2.415	20.325	1.00	20.18	A
ATOM	471	CB	ARG	165	-13.985	1.806	21.049	1.00	24.65	A
ATOM	472	CG	ARG	165	-14.363	0.833	22.126	1.00	29.54	A
ATOM	473	CD	ARG	165	-13.274	1.077	23.145	1.00	38.82	A
ATOM	474	NE	ARG	165	-13.719	1.998	24.186	1.00	43.41	A
ATOM	475	HE	ARG	165	-14.331	1.671	24.908	1.00	15.00	A
ATOM	476	CZ	ARG	165	-13.190	3.250	24.362	1.00	44.06	A
ATOM	477	NH1	ARG	165	-13.406	3.765	25.562	1.00	41.25	A
ATOM	478	HH11	ARG	165	-13.054	4.683	25.763	1.00	15.00	A
ATOM	479	HH12	ARG	165	-13.919	3.249	26.250	1.00	15.00	A

FIGURE 2I

ATOM	480	NH2	ARG	165	-12.485	3.946	23.425	1.00	31.65	A
ATOM	481	HH21	ARG	165	-12.133	4.860	23.623	1.00	15.00	A
ATOM	482	HH22	ARG	165	-12.322	3.527	22.530	1.00	15.00	A
ATOM	483	C	ARG	165	-14.608	3.554	19.510	1.00	17.70	A
ATOM	484	O	ARG	165	-14.018	3.450	18.441	1.00	18.26	A
ATOM	485	N	GLN	166	-14.763	4.687	20.151	1.00	17.43	A
ATOM	486	H	GLN	166	-15.263	4.614	21.007	1.00	15.00	A
ATOM	487	CA	GLN	166	-14.138	5.911	19.698	1.00	19.00	A
ATOM	488	CB	GLN	166	-14.613	7.021	20.610	1.00	23.79	A
ATOM	489	CG	GLN	166	-14.067	8.409	20.386	1.00	34.06	A
ATOM	490	CD	GLN	166	-15.178	9.399	20.659	1.00	45.91	A
ATOM	491	OE1	GLN	166	-15.102	10.492	20.135	1.00	53.64	A
ATOM	492	NE2	GLN	166	-16.202	9.046	21.418	1.00	44.10	A
ATOM	493	HE21	GLN	166	-16.906	9.765	21.443	1.00	15.00	A
ATOM	494	HE22	GLN	166	-16.577	8.287	21.935	1.00	15.00	A
ATOM	495	C	GLN	166	-12.649	5.881	19.644	1.00	17.48	A
ATOM	496	O	GLN	166	-12.029	5.378	20.561	1.00	18.13	A
ATOM	497	N	GLY	167	-12.160	6.478	18.565	1.00	14.83	A
ATOM	498	H	GLY	167	-12.750	6.836	17.850	1.00	15.00	A
ATOM	499	CA	GLY	167	-10.728	6.711	18.557	1.00	16.28	A
ATOM	500	C	GLY	167	-10.044	6.685	17.204	1.00	16.48	A
ATOM	501	O	GLY	167	-10.674	6.601	16.162	1.00	19.19	A
ATOM	502	N	LEU	168	-8.720	6.735	17.209	1.00	17.06	A
ATOM	503	H	LEU	168	-8.311	6.890	18.120	1.00	15.00	A
ATOM	504	CA	LEU	168	-7.925	6.625	15.992	1.00	16.60	A
ATOM	505	CB	LEU	168	-6.600	7.343	16.289	1.00	21.87	A
ATOM	506	CG	LEU	168	-6.247	8.745	15.716	1.00	22.69	A
ATOM	507	CD1	LEU	168	-5.119	9.410	16.539	1.00	21.20	A
ATOM	508	CD2	LEU	168	-7.436	9.617	15.361	1.00	18.38	A
ATOM	509	C	LEU	168	-7.686	5.136	15.604	1.00	14.84	A
ATOM	510	O	LEU	168	-7.282	4.278	16.392	1.00	15.89	A
ATOM	511	N	TYR	169	-7.943	4.873	14.300	1.00	10.57	A
ATOM	512	H	TYR	169	-8.313	5.659	13.807	1.00	15.00	A
ATOM	513	CA	TYR	169	-7.683	3.572	13.656	1.00	5.27	A
ATOM	514	CB	TYR	169	-8.989	3.014	13.230	1.00	5.83	A
ATOM	515	CG	TYR	169	-9.857	2.620	14.423	1.00	6.94	A
ATOM	516	CD1	TYR	169	-10.524	3.598	15.168	1.00	7.40	A
ATOM	517	CE1	TYR	169	-11.390	3.193	16.218	1.00	7.77	A
ATOM	518	CD2	TYR	169	-10.016	1.255	14.744	1.00	8.89	A
ATOM	519	CE2	TYR	169	-10.850	0.841	15.804	1.00	9.40	A
ATOM	520	CZ	TYR	169	-11.563	1.827	16.534	1.00	10.39	A
ATOM	521	OH	TYR	169	-12.443	1.410	17.534	1.00	7.99	A
ATOM	522	HH	TYR	169	-13.009	2.117	17.800	1.00	15.00	A
ATOM	523	C	TYR	169	-6.810	3.642	12.390	1.00	6.72	A
ATOM	524	O	TYR	169	-6.917	4.498	11.557	1.00	9.12	A
ATOM	525	N	TYR	170	-5.899	2.722	12.228	1.00	9.53	A
ATOM	526	H	TYR	170	-5.806	2.081	12.986	1.00	15.00	A
ATOM	527	CA	TYR	170	-5.313	2.511	10.899	1.00	10.01	A
ATOM	528	CB	TYR	170	-3.967	1.797	11.044	1.00	7.46	A
ATOM	529	CG	TYR	170	-3.259	1.636	9.679	1.00	13.45	A
ATOM	530	CD1	TYR	170	-2.680	2.766	9.052	1.00	12.66	A
ATOM	531	CE1	TYR	170	-2.213	2.658	7.738	1.00	10.18	A
ATOM	532	CD2	TYR	170	-3.304	0.385	9.057	1.00	10.90	A
ATOM	533	CE2	TYR	170	-2.891	0.303	7.730	1.00	8.68	A
ATOM	534	CZ	TYR	170	-2.331	1.419	7.124	1.00	9.97	A
ATOM	535	OH	TYR	170	-1.774	1.286	5.859	1.00	17.50	A
ATOM	536	HH	TYR	170	-1.886	0.404	5.514	1.00	15.00	A
ATOM	537	C	TYR	170	-6.279	1.610	10.073	1.00	10.40	A
ATOM	538	O	TYR	170	-6.679	0.500	10.421	1.00	12.52	A
ATOM	539	N	ILE	171	-6.704	2.174	8.968	1.00	12.16	A

FIGURE 2J

ATOM	540	H	ILE	171	-6.475	3.135	8.808	1.00	15.00	A
ATOM	541	CA	ILE	171	-7.608	1.430	8.138	1.00	9.37	A
ATOM	542	CB	ILE	171	-9.070	1.990	8.317	1.00	11.21	A
ATOM	543	CG2	ILE	171	-9.326	3.501	8.677	1.00	10.27	A
ATOM	544	CG1	ILE	171	-10.046	1.564	7.214	1.00	13.33	A
ATOM	545	CD1	ILE	171	-10.647	0.250	7.619	1.00	17.53	A
ATOM	546	C	ILE	171	-7.074	1.234	6.694	1.00	8.34	A
ATOM	547	O	ILE	171	-6.453	2.088	6.082	1.00	6.96	A
ATOM	548	N	TYR	172	-7.286	0.005	6.216	1.00	11.07	A
ATOM	549	H	TYR	172	-7.809	-0.624	6.786	1.00	15.00	A
ATOM	550	CA	TYR	172	-6.708	-0.378	4.922	1.00	15.60	A
ATOM	551	CB	TYR	172	-5.332	-1.082	5.037	1.00	14.32	A
ATOM	552	CG	TYR	172	-5.389	-2.397	5.796	1.00	9.21	A
ATOM	553	CD1	TYR	172	-5.342	-2.402	7.216	1.00	12.52	A
ATOM	554	CE1	TYR	172	-5.607	-3.620	7.901	1.00	10.88	A
ATOM	555	CD2	TYR	172	-5.565	-3.586	5.050	1.00	12.66	A
ATOM	556	CE2	TYR	172	-5.829	-4.800	5.740	1.00	15.83	A
ATOM	557	CZ	TYR	172	-5.822	-4.808	7.164	1.00	11.94	A
ATOM	558	OH	TYR	172	-5.995	-6.002	7.820	1.00	12.17	A
ATOM	559	HH	TYR	172	-6.433	-5.843	8.657	1.00	15.00	A
ATOM	560	C	TYR	172	-7.605	-1.276	4.106	1.00	16.85	A
ATOM	561	O	TYR	172	-8.346	-2.057	4.692	1.00	14.06	A
ATOM	562	N	ALA	173	-7.448	-1.141	2.776	1.00	16.29	A
ATOM	563	H	ALA	173	-6.751	-0.490	2.503	1.00	15.00	A
ATOM	564	CA	ALA	173	-7.940	-2.152	1.836	1.00	15.11	A
ATOM	565	CB	ALA	173	-9.300	-1.725	1.292	1.00	12.08	A
ATOM	566	C	ALA	173	-7.007	-2.537	0.653	1.00	15.86	A
ATOM	567	O	ALA	173	-6.147	-1.806	0.191	1.00	14.20	A
ATOM	568	N	GLN	174	-7.244	-3.714	0.109	1.00	16.56	A
ATOM	569	H	GLN	174	-7.774	-4.389	0.620	1.00	15.00	A
ATOM	570	CA	GLN	174	-6.470	-4.119	-1.070	1.00	19.25	A
ATOM	571	CB	GLN	174	-5.582	-5.292	-0.832	1.00	21.99	A
ATOM	572	CG	GLN	174	-4.205	-4.727	-1.030	1.00	30.99	A
ATOM	573	CD	GLN	174	-3.174	-5.845	-0.979	1.00	34.25	A
ATOM	574	OE1	GLN	174	-2.308	-5.899	-0.105	1.00	32.91	A
ATOM	575	NE2	GLN	174	-3.268	-6.699	-2.014	1.00	31.50	A
ATOM	576	HE21	GLN	174	-2.668	-7.487	-1.970	1.00	15.00	A
ATOM	577	HE22	GLN	174	-3.973	-6.621	-2.714	1.00	15.00	A
ATOM	578	C	GLN	174	-7.413	-4.644	-2.114	1.00	19.20	A
ATOM	579	O	GLN	174	-8.285	-5.434	-1.880	1.00	20.03	A
ATOM	580	N	VAL	175	-7.291	-4.107	-3.301	1.00	19.28	A
ATOM	581	H	VAL	175	-6.594	-3.401	-3.400	1.00	15.00	A
ATOM	582	CA	VAL	175	-8.247	-4.500	-4.323	1.00	22.43	A
ATOM	583	CB	VAL	175	-9.319	-3.409	-4.644	1.00	21.41	A
ATOM	584	CG1	VAL	175	-10.146	-2.830	-3.495	1.00	20.17	A
ATOM	585	CG2	VAL	175	-10.268	-4.061	-5.639	1.00	22.88	A
ATOM	586	C	VAL	175	-7.508	-4.859	-5.615	1.00	24.56	A
ATOM	587	O	VAL	175	-6.928	-3.997	-6.301	1.00	23.28	A
ATOM	588	N	THR	176	-7.563	-6.180	-5.879	1.00	25.40	A
ATOM	589	H	THR	176	-7.994	-6.850	-5.250	1.00	15.00	A
ATOM	590	CA	THR	176	-7.086	-6.501	-7.222	1.00	24.46	A
ATOM	591	CB	THR	176	-5.844	-7.454	-7.256	1.00	24.78	A
ATOM	592	OG1	THR	176	-5.948	-8.650	-8.028	1.00	20.31	A
ATOM	593	HG1	THR	176	-5.250	-9.253	-7.796	1.00	15.00	A
ATOM	594	CG2	THR	176	-5.329	-7.711	-5.867	1.00	17.07	A
ATOM	595	C	THR	176	-8.178	-6.700	-8.272	1.00	25.44	A
ATOM	596	O	THR	176	-9.326	-7.043	-7.995	1.00	26.86	A
ATOM	597	N	PHE	177	-7.855	-6.341	-9.506	1.00	22.44	A
ATOM	598	H	PHE	177	-6.920	-6.083	-9.732	1.00	15.00	A
ATOM	599	CA	PHE	177	-8.939	-6.511	-10.479	1.00	22.70	A

FIGURE 2K

ATOM	600	CB	PHE	177	-9.746	-5.194	-10.599	1.00	20.90	A
ATOM	601	CG	PHE	177	-8.813	-4.034	-10.927	1.00	22.51	A
ATOM	602	CD1	PHE	177	-8.771	-3.548	-12.252	1.00	22.11	A
ATOM	603	CD2	PHE	177	-8.011	-3.422	-9.920	1.00	21.87	A
ATOM	604	CE1	PHE	177	-8.041	-2.387	-12.550	1.00	20.53	A
ATOM	605	CE2	PHE	177	-7.289	-2.247	-10.204	1.00	20.44	A
ATOM	606	CZ	PHE	177	-7.376	-1.713	-11.500	1.00	22.79	A
ATOM	607	C	PHE	177	-8.381	-6.949	-11.800	1.00	22.14	A
ATOM	608	O	PHE	177	-7.219	-6.695	-12.072	1.00	21.60	A
ATOM	609	N	CYS	178	-9.210	-7.555	-12.625	1.00	24.52	A
ATOM	610	H	CYS	178	-10.146	-7.797	-12.370	1.00	15.00	A
ATOM	611	CA	CYS	178	-8.599	-7.849	-13.942	1.00	29.77	A
ATOM	612	CB	CYS	178	-8.501	-9.365	-14.214	1.00	32.06	A
ATOM	613	SG	CYS	178	-7.685	-9.731	-15.792	1.00	35.17	A
ATOM	614	C	CYS	178	-9.323	-7.146	-15.088	1.00	28.41	A
ATOM	615	O	CYS	178	-10.534	-7.247	-15.185	1.00	27.54	A
ATOM	616	N	SER	179	-8.589	-6.393	-15.910	1.00	28.86	A
ATOM	617	H	SER	179	-7.608	-6.271	-15.754	1.00	15.00	A
ATOM	618	CA	SER	179	-9.374	-5.454	-16.704	1.00	29.01	A
ATOM	619	CB	SER	179	-9.379	-4.118	-16.020	1.00	30.82	A
ATOM	620	OG	SER	179	-10.615	-3.492	-16.319	1.00	39.79	A
ATOM	621	HG	SER	179	-10.725	-2.812	-15.667	1.00	15.00	A
ATOM	622	C	SER	179	-9.063	-5.196	-18.165	1.00	31.16	A
ATOM	623	O	SER	179	-7.931	-4.953	-18.537	1.00	28.58	A
ATOM	624	N	ASN	180	-10.083	-5.255	-19.042	1.00	35.32	A
ATOM	625	H	ASN	180	-10.966	-5.700	-18.834	1.00	15.00	A
ATOM	626	CA	ASN	180	-9.782	-4.725	-20.366	1.00	34.74	A
ATOM	627	CB	ASN	180	-10.205	-5.554	-21.589	1.00	37.96	A
ATOM	628	CG	ASN	180	-9.650	-4.980	-22.896	1.00	37.12	A
ATOM	629	OD1	ASN	180	-10.058	-3.947	-23.356	1.00	40.66	A
ATOM	630	ND2	ASN	180	-8.619	-5.536	-23.456	1.00	35.85	A
ATOM	631	HD21	ASN	180	-8.343	-6.475	-23.306	1.00	15.00	A
ATOM	632	HD22	ASN	180	-8.153	-4.891	-24.065	1.00	15.00	A
ATOM	633	C	ASN	180	-10.197	-3.331	-20.588	1.00	36.96	A
ATOM	634	O	ASN	180	-11.314	-2.894	-20.433	1.00	37.89	A
ATOM	635	N	ARG	181	-9.147	-2.699	-21.068	1.00	41.95	A
ATOM	636	H	ARG	181	-8.363	-3.318	-21.141	1.00	15.00	A
ATOM	637	CA	ARG	181	-8.997	-1.313	-21.489	1.00	44.24	A
ATOM	638	CB	ARG	181	-7.563	-1.279	-22.026	1.00	43.43	A
ATOM	639	CG	ARG	181	-6.348	-1.638	-21.101	1.00	45.11	A
ATOM	640	CD	ARG	181	-6.235	-2.853	-20.134	1.00	40.68	A
ATOM	641	NE	ARG	181	-5.064	-2.772	-19.271	1.00	46.11	A
ATOM	642	HE	ARG	181	-4.991	-2.058	-18.578	1.00	15.00	A
ATOM	643	CZ	ARG	181	-4.024	-3.611	-19.432	1.00	49.77	A
ATOM	644	NH1	ARG	181	-2.886	-3.414	-18.790	1.00	54.33	A
ATOM	645	HH11	ARG	181	-2.113	-4.032	-18.918	1.00	15.00	A
ATOM	646	HH12	ARG	181	-2.807	-2.642	-18.161	1.00	15.00	A
ATOM	647	NH2	ARG	181	-4.085	-4.641	-20.247	1.00	54.26	A
ATOM	648	HH21	ARG	181	-3.286	-5.230	-20.354	1.00	15.00	A
ATOM	649	HH22	ARG	181	-4.918	-4.833	-20.761	1.00	15.00	A
ATOM	650	C	ARG	181	-10.049	-0.866	-22.499	1.00	47.10	A
ATOM	651	O	ARG	181	-10.979	-0.112	-22.227	1.00	49.20	A
ATOM	652	N	GLU	182	-9.895	-1.447	-23.690	1.00	49.64	A
ATOM	653	H	GLU	182	-9.201	-2.166	-23.775	1.00	15.00	A
ATOM	654	CA	GLU	182	-10.976	-1.385	-24.676	1.00	52.41	A
ATOM	655	CB	GLU	182	-10.437	-2.020	-25.970	1.00	56.93	A
ATOM	656	CG	GLU	182	-10.932	-1.418	-27.295	1.00	66.05	A
ATOM	657	CD	GLU	182	-10.758	0.116	-27.327	1.00	70.54	A
ATOM	658	OE1	GLU	182	-9.613	0.586	-27.442	1.00	72.98	A
ATOM	659	OE2	GLU	182	-11.778	0.830	-27.244	1.00	72.46	A

FIGURE 2L

ATOM	660	C	GLU	182	-12.388	-1.934	-24.304	1.00	53.00	A
ATOM	661	O	GLU	182	-13.379	-1.492	-24.862	1.00	54.27	A
ATOM	662	N	ALA	183	-12.505	-2.877	-23.335	1.00	52.34	A
ATOM	663	H	ALA	183	-11.676	-3.173	-22.865	1.00	15.00	A
ATOM	664	CA	ALA	183	-13.867	-3.258	-22.899	1.00	50.19	A
ATOM	665	CB	ALA	183	-13.855	-4.721	-22.447	1.00	45.02	A
ATOM	666	C	ALA	183	-14.562	-2.321	-21.867	1.00	50.66	A
ATOM	667	O	ALA	183	-15.712	-1.945	-21.990	1.00	47.77	A
ATOM	668	N	SER	184	-13.773	-1.888	-20.878	1.00	52.95	A
ATOM	669	H	SER	184	-12.826	-2.172	-20.991	1.00	15.00	A
ATOM	670	CA	SER	184	-14.228	-1.043	-19.729	1.00	56.78	A
ATOM	671	CB	SER	184	-13.384	-1.397	-18.481	1.00	53.58	A
ATOM	672	OG	SER	184	-13.975	-2.448	-17.721	1.00	47.46	A
ATOM	673	HG	SER	184	-13.291	-3.019	-17.388	1.00	15.00	A
ATOM	674	C	SER	184	-14.183	0.517	-19.880	1.00	59.95	A
ATOM	675	O	SER	184	-13.913	1.297	-18.964	1.00	65.25	A
ATOM	676	N	SER	185	-14.324	0.995	-21.131	1.00	60.08	A
ATOM	677	H	SER	185	-14.623	0.345	-21.831	1.00	15.00	A
ATOM	678	CA	SER	185	-13.825	2.375	-21.391	1.00	60.12	A
ATOM	679	CB	SER	185	-13.522	2.640	-22.869	1.00	60.49	A
ATOM	680	OG	SER	185	-12.243	2.098	-23.242	1.00	59.80	A
ATOM	681	HG	SER	185	-12.158	1.234	-22.833	1.00	15.00	A
ATOM	682	C	SER	185	-14.580	3.589	-20.885	1.00	59.59	A
ATOM	683	O	SER	185	-15.437	4.159	-21.543	1.00	60.08	A
ATOM	684	N	GLN	186	-14.200	3.990	-19.670	1.00	57.71	A
ATOM	685	H	GLN	186	-13.601	3.376	-19.153	1.00	15.00	A
ATOM	686	CA	GLN	186	-15.121	4.936	-18.993	1.00	57.00	A
ATOM	687	CB	GLN	186	-16.094	4.062	-18.175	1.00	58.66	A
ATOM	688	CG	GLN	186	-15.355	3.354	-17.050	1.00	59.69	A
ATOM	689	CD	GLN	186	-16.369	2.789	-16.088	1.00	59.92	A
ATOM	690	OE1	GLN	186	-17.270	3.513	-15.687	1.00	59.81	A
ATOM	691	NE2	GLN	186	-16.249	1.503	-15.787	1.00	59.63	A
ATOM	692	HE21	GLN	186	-15.492	0.948	-16.113	1.00	15.00	A
ATOM	693	HE22	GLN	186	-16.950	1.119	-15.168	1.00	15.00	A
ATOM	694	C	GLN	186	-14.758	6.290	-18.221	1.00	54.36	A
ATOM	695	O	GLN	186	-15.596	7.198	-18.298	1.00	53.98	A
ATOM	696	N	ALA	187	-13.566	6.424	-17.511	1.00	50.35	A
ATOM	697	H	ALA	187	-13.476	7.274	-16.970	1.00	15.00	A
ATOM	698	CA	ALA	187	-12.388	5.599	-17.832	1.00	43.26	A
ATOM	699	CB	ALA	187	-11.546	6.284	-18.918	1.00	38.95	A
ATOM	700	C	ALA	187	-11.456	4.882	-16.849	1.00	40.48	A
ATOM	701	O	ALA	187	-10.887	3.875	-17.295	1.00	43.24	A
ATOM	702	N	PRO	188	-11.210	5.383	-15.594	1.00	38.66	A
ATOM	703	CD	PRO	188	-11.543	6.687	-15.000	1.00	38.15	A
ATOM	704	CA	PRO	188	-10.220	4.665	-14.751	1.00	35.94	A
ATOM	705	CB	PRO	188	-9.395	5.813	-14.150	1.00	33.99	A
ATOM	706	CG	PRO	188	-10.377	7.000	-14.036	1.00	32.69	A
ATOM	707	C	PRO	188	-10.840	3.783	-13.683	1.00	33.66	A
ATOM	708	O	PRO	188	-11.885	4.062	-13.140	1.00	33.41	A
ATOM	709	N	PHE	189	-10.147	2.695	-13.346	1.00	28.66	A
ATOM	710	H	PHE	189	-9.260	2.508	-13.748	1.00	15.00	A
ATOM	711	CA	PHE	189	-10.721	2.013	-12.171	1.00	26.71	A
ATOM	712	CB	PHE	189	-10.122	0.601	-12.034	1.00	26.21	A
ATOM	713	CG	PHE	189	-10.671	-0.189	-10.849	1.00	22.92	A
ATOM	714	CD1	PHE	189	-10.126	0.005	-9.566	1.00	17.72	A
ATOM	715	CD2	PHE	189	-11.687	-1.165	-11.064	1.00	21.88	A
ATOM	716	CE1	PHE	189	-10.590	-0.815	-8.522	1.00	19.12	A
ATOM	717	CE2	PHE	189	-12.124	-1.995	-10.011	1.00	21.13	A
ATOM	718	CZ	PHE	189	-11.571	-1.806	-8.736	1.00	18.44	A
ATOM	719	C	PHE	189	-10.445	2.815	-10.909	1.00	27.14	A

FIGURE 2M

ATOM	720	O	PHE	189	-9.308	3.244	-10.706	1.00	28.72	A
ATOM	721	N	ILE	190	-11.468	2.964	-10.071	1.00	24.71	A
ATOM	722	H	ILE	190	-12.408	2.786	-10.388	1.00	15.00	A
ATOM	723	CA	ILE	190	-11.193	3.626	-8.788	1.00	24.03	A
ATOM	724	CB	ILE	190	-11.316	5.242	-8.743	1.00	26.86	A
ATOM	725	CG2	ILE	190	-11.892	5.979	-9.997	1.00	19.87	A
ATOM	726	CG1	ILE	190	-11.801	5.888	-7.424	1.00	22.54	A
ATOM	727	CD1	ILE	190	-12.819	7.012	-7.645	1.00	28.56	A
ATOM	728	C	ILE	190	-11.844	2.812	-7.656	1.00	21.97	A
ATOM	729	O	ILE	190	-12.891	2.197	-7.801	1.00	16.30	A
ATOM	730	N	ALA	191	-11.026	2.700	-6.590	1.00	17.21	A
ATOM	731	H	ALA	191	-10.124	3.124	-6.662	1.00	15.00	A
ATOM	732	CA	ALA	191	-11.501	2.195	-5.321	1.00	15.20	A
ATOM	733	CB	ALA	191	-10.730	0.928	-4.968	1.00	14.79	A
ATOM	734	C	ALA	191	-11.439	3.230	-4.206	1.00	17.11	A
ATOM	735	O	ALA	191	-10.467	3.961	-4.052	1.00	14.04	A
ATOM	736	N	SER	192	-12.511	3.245	-3.433	1.00	14.72	A
ATOM	737	H	SER	192	-13.277	2.694	-3.804	1.00	15.00	A
ATOM	738	CA	SER	192	-12.725	4.289	-2.423	1.00	16.69	A
ATOM	739	CB	SER	192	-13.931	5.144	-2.803	1.00	14.83	A
ATOM	740	OG	SER	192	-13.556	5.828	-3.994	1.00	21.23	A
ATOM	741	HG	SER	192	-14.367	5.966	-4.520	1.00	15.00	A
ATOM	742	C	SER	192	-12.980	3.682	-1.069	1.00	17.77	A
ATOM	743	O	SER	192	-13.753	2.738	-0.947	1.00	20.76	A
ATOM	744	N	LEU	193	-12.285	4.209	-0.038	1.00	15.56	A
ATOM	745	H	LEU	193	-11.681	4.959	-0.280	1.00	15.00	A
ATOM	746	CA	LEU	193	-12.510	3.761	1.366	1.00	13.27	A
ATOM	747	CB	LEU	193	-11.195	3.825	2.217	1.00	12.74	A
ATOM	748	CG	LEU	193	-11.051	3.141	3.604	1.00	14.37	A
ATOM	749	CD1	LEU	193	-12.272	2.354	4.116	1.00	14.67	A
ATOM	750	CD2	LEU	193	-10.274	3.986	4.622	1.00	12.64	A
ATOM	751	C	LEU	193	-13.497	4.748	1.911	1.00	11.22	A
ATOM	752	O	LEU	193	-13.188	5.912	1.903	1.00	12.22	A
ATOM	753	N	CYS	194	-14.652	4.326	2.310	1.00	13.66	A
ATOM	754	H	CYS	194	-14.828	3.347	2.276	1.00	15.00	A
ATOM	755	CA	CYS	194	-15.595	5.360	2.713	1.00	14.84	A
ATOM	756	CB	CYS	194	-16.915	5.409	1.918	1.00	17.58	A
ATOM	757	SG	CYS	194	-16.623	5.417	0.165	1.00	16.33	A
ATOM	758	C	CYS	194	-16.046	5.163	4.137	1.00	12.81	A
ATOM	759	O	CYS	194	-15.983	4.072	4.655	1.00	10.34	A
ATOM	760	N	LEU	195	-16.557	6.254	4.697	1.00	14.32	A
ATOM	761	H	LEU	195	-16.541	7.088	4.154	1.00	15.00	A
ATOM	762	CA	LEU	195	-17.039	6.291	6.076	1.00	14.89	A
ATOM	763	CB	LEU	195	-16.195	7.372	6.789	1.00	15.56	A
ATOM	764	CG	LEU	195	-16.571	7.680	8.242	1.00	15.56	A
ATOM	765	CD1	LEU	195	-15.932	8.967	8.762	1.00	13.72	A
ATOM	766	CD2	LEU	195	-16.463	6.448	9.154	1.00	17.25	A
ATOM	767	C	LEU	195	-18.546	6.544	6.209	1.00	13.54	A
ATOM	768	O	LEU	195	-19.038	7.521	5.705	1.00	14.56	A
ATOM	769	N	LYS	196	-19.238	5.667	6.905	1.00	16.36	A
ATOM	770	H	LYS	196	-18.719	4.875	7.197	1.00	15.00	A
ATOM	771	CA	LYS	196	-20.577	5.972	7.405	1.00	21.01	A
ATOM	772	CB	LYS	196	-21.475	4.726	7.146	1.00	22.66	A
ATOM	773	CG	LYS	196	-22.953	4.839	7.590	1.00	31.25	A
ATOM	774	CD	LYS	196	-23.364	4.915	9.104	1.00	40.25	A
ATOM	775	CE	LYS	196	-23.189	3.694	10.060	1.00	43.56	A
ATOM	776	NZ	LYS	196	-23.004	4.158	11.453	1.00	44.46	A
ATOM	777	HZ1	LYS	196	-22.182	4.799	11.467	1.00	15.00	A
ATOM	778	HZ2	LYS	196	-23.847	4.665	11.778	1.00	15.00	A
ATOM	779	HZ3	LYS	196	-22.807	3.334	12.066	1.00	15.00	A

FIGURE 2N

ATOM	780	C	LYS	196	-20.478	6.290	8.899	1.00	19.25	A
ATOM	781	O	LYS	196	-20.194	5.434	9.714	1.00	18.35	A
ATOM	782	N	SER	197	-20.664	7.534	9.272	1.00	20.63	A
ATOM	783	H	SER	197	-20.891	8.247	8.615	1.00	15.00	A
ATOM	784	CA	SER	197	-20.752	7.701	10.729	1.00	24.87	A
ATOM	785	CB	SER	197	-19.898	8.878	11.207	1.00	25.62	A
ATOM	786	OG	SER	197	-19.563	8.687	12.588	1.00	32.22	A
ATOM	787	HG	SER	197	-18.795	8.110	12.611	1.00	15.00	A
ATOM	788	C	SER	197	-22.216	7.810	11.218	1.00	26.33	A
ATOM	789	O	SER	197	-23.078	8.303	10.497	1.00	26.57	A
ATOM	790	N	PRO	198	-22.534	7.274	12.407	1.00	26.77	A
ATOM	791	CD	PRO	198	-21.649	6.526	13.301	1.00	32.92	A
ATOM	792	CA	PRO	198	-23.919	7.381	12.913	1.00	28.73	A
ATOM	793	CB	PRO	198	-23.784	6.789	14.318	1.00	32.89	A
ATOM	794	CG	PRO	198	-22.289	6.726	14.659	1.00	33.55	A
ATOM	795	C	PRO	198	-24.591	8.789	12.847	1.00	26.60	A
ATOM	796	O	PRO	198	-24.035	9.817	13.242	1.00	20.20	A
ATOM	797	N	GLY	199	-25.729	8.773	12.119	1.00	25.75	A
ATOM	798	H	GLY	199	-26.170	7.857	12.057	1.00	15.00	A
ATOM	799	CA	GLY	199	-26.486	10.003	11.790	1.00	26.91	A
ATOM	800	C	GLY	199	-25.821	10.971	10.816	1.00	28.98	A
ATOM	801	O	GLY	199	-26.084	12.151	10.797	1.00	31.05	A
ATOM	802	N	ARG	200	-24.898	10.464	10.001	1.00	30.15	A
ATOM	803	H	ARG	200	-24.629	9.519	10.165	1.00	15.00	A
ATOM	804	CA	ARG	200	-24.140	11.384	9.166	1.00	28.98	A
ATOM	805	CB	ARG	200	-22.749	11.590	9.783	1.00	33.16	A
ATOM	806	CG	ARG	200	-22.739	12.290	11.162	1.00	38.34	A
ATOM	807	CD	ARG	200	-21.327	12.530	11.705	1.00	42.14	A
ATOM	808	NE	ARG	200	-21.292	12.875	13.131	1.00	43.64	A
ATOM	809	HE	ARG	200	-21.327	13.831	13.424	1.00	15.00	A
ATOM	810	CZ	ARG	200	-21.138	11.896	14.051	1.00	46.40	A
ATOM	811	NH1	ARG	200	-21.219	10.603	13.733	1.00	46.31	A
ATOM	812	HH11	ARG	200	-21.104	9.910	14.445	1.00	15.00	A
ATOM	813	HH12	ARG	200	-21.394	10.320	12.789	1.00	15.00	A
ATOM	814	NH2	ARG	200	-20.901	12.226	15.311	1.00	46.65	A
ATOM	815	HH21	ARG	200	-20.847	13.193	15.566	1.00	15.00	A
ATOM	816	HH22	ARG	200	-20.785	11.510	16.002	1.00	15.00	A
ATOM	817	C	ARG	200	-24.084	10.967	7.710	1.00	27.77	A
ATOM	818	O	ARG	200	-24.264	9.791	7.449	1.00	28.21	A
ATOM	819	N	PHE	201	-23.853	11.926	6.792	1.00	30.83	A
ATOM	820	H	PHE	201	-23.513	12.821	7.126	1.00	15.00	A
ATOM	821	CA	PHE	201	-24.016	11.708	5.339	1.00	34.17	A
ATOM	822	CB	PHE	201	-23.851	12.996	4.572	1.00	31.58	A
ATOM	823	CG	PHE	201	-25.154	13.730	4.614	1.00	34.85	A
ATOM	824	CD1	PHE	201	-25.174	15.062	5.081	1.00	37.56	A
ATOM	825	CD2	PHE	201	-26.335	13.081	4.190	1.00	37.89	A
ATOM	826	CE1	PHE	201	-26.397	15.749	5.182	1.00	36.91	A
ATOM	827	CE2	PHE	201	-27.566	13.762	4.280	1.00	38.98	A
ATOM	828	CZ	PHE	201	-27.572	15.065	4.815	1.00	37.61	A
ATOM	829	C	PHE	201	-23.277	10.605	4.545	1.00	39.40	A
ATOM	830	O	PHE	201	-23.853	10.034	3.604	1.00	45.71	A
ATOM	831	N	GLU	202	-22.031	10.316	5.034	1.00	35.75	A
ATOM	832	H	GLU	202	-21.878	10.753	5.925	1.00	15.00	A
ATOM	833	CA	GLU	202	-20.964	9.564	4.318	1.00	34.52	A
ATOM	834	CB	GLU	202	-21.295	8.540	3.234	1.00	33.66	A
ATOM	835	CG	GLU	202	-21.924	7.245	3.713	1.00	40.61	A
ATOM	836	CD	GLU	202	-22.647	6.505	2.561	1.00	46.12	A
ATOM	837	OE1	GLU	202	-23.461	5.613	2.886	1.00	46.89	A
ATOM	838	OE2	GLU	202	-22.417	6.814	1.370	1.00	45.63	A
ATOM	839	C	GLU	202	-19.924	10.450	3.717	1.00	29.99	A

FIGURE 2O

ATOM	840	O	GLU	202	-20.137	11.567	3.300	1.00	30.76	A
ATOM	841	N	ARG	203	-18.728	9.897	3.856	1.00	26.88	A
ATOM	842	H	ARG	203	-18.690	8.998	4.285	1.00	15.00	A
ATOM	843	CA	ARG	203	-17.539	10.603	3.358	1.00	21.88	A
ATOM	844	CB	ARG	203	-16.819	11.410	4.457	1.00	27.07	A
ATOM	845	CG	ARG	203	-17.681	12.187	5.467	1.00	37.32	A
ATOM	846	CD	ARG	203	-16.894	13.213	6.339	1.00	48.09	A
ATOM	847	NE	ARG	203	-15.911	12.667	7.308	1.00	56.90	A
ATOM	848	HE	ARG	203	-16.240	12.433	8.223	1.00	15.00	A
ATOM	849	CZ	ARG	203	-14.572	12.475	7.001	1.00	66.77	A
ATOM	850	NH1	ARG	203	-13.702	12.002	7.911	1.00	68.44	A
ATOM	851	HH11	ARG	203	-12.745	11.829	7.666	1.00	15.00	A
ATOM	852	HH12	ARG	203	-14.016	11.822	8.845	1.00	15.00	A
ATOM	853	NH2	ARG	203	-14.084	12.716	5.766	1.00	67.68	A
ATOM	854	HH21	ARG	203	-14.670	13.108	5.060	1.00	15.00	A
ATOM	855	HH22	ARG	203	-13.143	12.499	5.544	1.00	15.00	A
ATOM	856	C	ARG	203	-16.517	9.633	2.678	1.00	17.71	A
ATOM	857	O	ARG	203	-16.375	8.418	2.931	1.00	7.69	A
ATOM	858	N	ILE	204	-15.789	10.253	1.791	1.00	14.42	A
ATOM	859	H	ILE	204	-15.915	11.228	1.561	1.00	15.00	A
ATOM	860	CA	ILE	204	-14.662	9.482	1.353	1.00	18.32	A
ATOM	861	CB	ILE	204	-14.520	9.392	-0.231	1.00	24.52	A
ATOM	862	CG2	ILE	204	-15.820	9.529	-1.069	1.00	21.85	A
ATOM	863	CG1	ILE	204	-13.439	10.195	-0.949	1.00	26.35	A
ATOM	864	CD1	ILE	204	-13.992	11.231	-1.961	1.00	36.33	A
ATOM	865	C	ILE	204	-13.387	9.819	2.153	1.00	16.58	A
ATOM	866	O	ILE	204	-13.070	10.956	2.457	1.00	18.63	A
ATOM	867	N	LEU	205	-12.718	8.725	2.571	1.00	13.32	A
ATOM	868	H	LEU	205	-13.142	7.853	2.321	1.00	15.00	A
ATOM	869	CA	LEU	205	-11.467	8.829	3.322	1.00	10.01	A
ATOM	870	CB	LEU	205	-11.440	7.688	4.382	1.00	6.66	A
ATOM	871	CG	LEU	205	-12.571	7.727	5.441	1.00	7.99	A
ATOM	872	CD1	LEU	205	-12.722	9.088	6.089	1.00	8.78	A
ATOM	873	CD2	LEU	205	-12.419	6.720	6.582	1.00	8.08	A
ATOM	874	C	LEU	205	-10.268	8.811	2.377	1.00	9.75	A
ATOM	875	O	LEU	205	-9.416	9.655	2.320	1.00	10.25	A
ATOM	876	N	LEU	206	-10.252	7.769	1.562	1.00	10.28	A
ATOM	877	H	LEU	206	-10.991	7.119	1.684	1.00	15.00	A
ATOM	878	CA	LEU	206	-9.166	7.555	0.610	1.00	10.02	A
ATOM	879	CB	LEU	206	-8.249	6.384	0.990	1.00	11.94	A
ATOM	880	CG	LEU	206	-7.001	6.527	1.859	1.00	14.40	A
ATOM	881	CD1	LEU	206	-7.094	5.595	3.074	1.00	14.49	A
ATOM	882	CD2	LEU	206	-6.531	7.958	2.151	1.00	8.78	A
ATOM	883	C	LEU	206	-9.756	7.071	-0.697	1.00	11.91	A
ATOM	884	O	LEU	206	-10.792	6.406	-0.778	1.00	10.67	A
ATOM	885	N	ARG	207	-9.005	7.428	-1.720	1.00	8.06	A
ATOM	886	H	ARG	207	-8.196	7.992	-1.553	1.00	15.00	A
ATOM	887	CA	ARG	207	-9.309	6.823	-2.992	1.00	10.45	A
ATOM	888	CB	ARG	207	-9.974	7.790	-3.904	1.00	8.71	A
ATOM	889	CG	ARG	207	-11.258	8.270	-3.357	1.00	15.68	A
ATOM	890	CD	ARG	207	-11.652	9.459	-4.163	1.00	22.25	A
ATOM	891	NE	ARG	207	-12.670	9.192	-5.171	1.00	29.59	A
ATOM	892	HE	ARG	207	-13.115	8.300	-5.249	1.00	15.00	A
ATOM	893	CZ	ARG	207	-13.063	10.272	-5.919	1.00	40.09	A
ATOM	894	NH1	ARG	207	-12.482	11.498	-5.813	1.00	36.32	A
ATOM	895	HH11	ARG	207	-12.813	12.246	-6.391	1.00	15.00	A
ATOM	896	HH12	ARG	207	-11.737	11.651	-5.165	1.00	15.00	A
ATOM	897	NH2	ARG	207	-14.067	10.111	-6.773	1.00	40.86	A
ATOM	898	HH21	ARG	207	-14.392	10.877	-7.329	1.00	15.00	A
ATOM	899	HH22	ARG	207	-14.498	9.207	-6.853	1.00	15.00	A

FIGURE 2P

ATOM	900	C	ARG	207	-8.044	6.456	-3.741	1.00	12.59	A
ATOM	901	O	ARG	207	-7.053	7.150	-3.787	1.00	15.58	A
ATOM	902	N	ALA	208	-8.096	5.358	-4.465	1.00	17.06	A
ATOM	903	H	ALA	208	-8.879	4.758	-4.355	1.00	15.00	A
ATOM	904	CA	ALA	208	-7.025	5.128	-5.465	1.00	17.00	A
ATOM	905	CB	ALA	208	-6.052	4.020	-5.072	1.00	14.69	A
ATOM	906	C	ALA	208	-7.544	4.830	-6.854	1.00	20.46	A
ATOM	907	O	ALA	208	-8.438	4.020	-7.057	1.00	21.89	A
ATOM	908	N	ALA	209	-6.986	5.586	-7.808	1.00	26.22	A
ATOM	909	H	ALA	209	-6.280	6.235	-7.533	1.00	15.00	A
ATOM	910	CA	ALA	209	-7.253	5.208	-9.196	1.00	28.06	A
ATOM	911	CB	ALA	209	-7.702	6.380	-10.069	1.00	27.10	A
ATOM	912	C	ALA	209	-6.075	4.461	-9.832	1.00	32.54	A
ATOM	913	O	ALA	209	-4.895	4.726	-9.593	1.00	33.00	A
ATOM	914	N	ASN	210	-6.502	3.491	-10.634	1.00	32.11	A
ATOM	915	H	ASN	210	-7.466	3.249	-10.531	1.00	15.00	A
ATOM	916	CA	ASN	210	-5.674	2.893	-11.662	1.00	36.00	A
ATOM	917	CB	ASN	210	-5.366	1.446	-11.355	1.00	39.53	A
ATOM	918	CG	ASN	210	-4.463	1.366	-10.154	1.00	42.59	A
ATOM	919	OD1	ASN	210	-4.285	2.273	-9.342	1.00	39.26	A
ATOM	920	ND2	ASN	210	-3.951	0.165	-10.055	1.00	41.77	A
ATOM	921	HD21	ASN	210	-3.990	-0.479	-10.817	1.00	15.00	A
ATOM	922	HD22	ASN	210	-3.364	-0.081	-9.279	1.00	15.00	A
ATOM	923	C	ASN	210	-6.299	2.931	-13.043	1.00	36.95	A
ATOM	924	O	ASN	210	-7.492	2.752	-13.259	1.00	36.93	A
ATOM	925	N	THR	211	-5.447	3.168	-14.013	1.00	37.83	A
ATOM	926	H	THR	211	-4.484	3.377	-13.821	1.00	15.00	A
ATOM	927	CA	THR	211	-6.119	3.224	-15.314	1.00	41.27	A
ATOM	928	CB	THR	211	-5.325	4.158	-16.268	1.00	44.53	A
ATOM	929	OG1	THR	211	-6.076	4.506	-17.438	1.00	49.34	A
ATOM	930	HG1	THR	211	-6.032	5.493	-17.508	1.00	15.00	A
ATOM	931	CG2	THR	211	-3.926	3.604	-16.581	1.00	46.08	A
ATOM	932	C	THR	211	-6.434	1.833	-15.878	1.00	39.17	A
ATOM	933	O	THR	211	-5.822	0.863	-15.475	1.00	36.48	A
ATOM	934	N	HIS	212	-7.416	1.718	-16.789	1.00	37.14	A
ATOM	935	H	HIS	212	-8.106	2.438	-16.878	1.00	15.00	A
ATOM	936	CA	HIS	212	-7.294	0.454	-17.529	1.00	33.23	A
ATOM	937	CB	HIS	212	-8.680	-0.012	-18.082	1.00	27.73	A
ATOM	938	CG	HIS	212	-9.856	0.060	-17.111	1.00	24.58	A
ATOM	939	ND1	HIS	212	-10.862	0.967	-17.161	1.00	24.59	A
ATOM	940	HD1	HIS	212	-11.000	1.702	-17.794	1.00	15.00	A
ATOM	941	CD2	HIS	212	-10.049	-0.723	-15.985	1.00	20.65	A
ATOM	942	NE2	HIS	212	-11.154	-0.265	-15.383	1.00	24.01	A
ATOM	943	CE1	HIS	212	-11.665	0.780	-16.092	1.00	17.59	A
ATOM	944	C	HIS	212	-6.257	0.633	-18.683	1.00	38.31	A
ATOM	945	O	HIS	212	-5.363	-0.132	-18.923	1.00	33.92	A
ATOM	946	N	SER	213	-6.444	1.737	-19.443	1.00	46.63	A
ATOM	947	H	SER	213	-7.156	2.323	-19.055	1.00	15.00	A
ATOM	948	CA	SER	213	-5.705	2.177	-20.675	1.00	53.91	A
ATOM	949	CB	SER	213	-4.272	2.704	-20.400	1.00	52.61	A
ATOM	950	OG	SER	213	-3.266	1.697	-20.547	1.00	53.97	A
ATOM	951	HG	SER	213	-3.363	1.064	-19.823	1.00	15.00	A
ATOM	952	C	SER	213	-5.844	1.508	-22.097	1.00	60.03	A
ATOM	953	O	SER	213	-6.005	0.811	-22.682	1.00	61.19	A
ATOM	954	N	SER	214	-7.043	1.803	-22.686	1.00	64.96	A
ATOM	955	H	SER	214	-7.705	2.322	-22.146	1.00	15.00	A
ATOM	956	CA	SER	214	-7.463	1.456	-24.094	1.00	69.62	A
ATOM	957	CB	SER	214	-8.727	2.218	-24.495	1.00	67.82	A
ATOM	958	OG	SER	214	-9.563	2.257	-23.336	1.00	67.64	A
ATOM	959	HG	SER	214	-10.468	2.398	-23.623	1.00	15.00	A

FIGURE 2Q

ATOM	960	C	SER	214	-6.518	1.587	-25.300	1.00	72.08	A
ATOM	961	O	SER	214	-6.102	2.683	-25.686	1.00	73.45	A
ATOM	962	N	ALA	215	-6.175	0.409	-25.899	1.00	73.36	A
ATOM	963	H	ALA	215	-5.456	0.596	-26.565	1.00	15.00	A
ATOM	964	CA	ALA	215	-6.858	-0.915	-25.753	1.00	72.62	A
ATOM	965	CB	ALA	215	-7.199	-1.505	-27.138	1.00	73.08	A
ATOM	966	C	ALA	215	-6.331	-2.148	-24.983	1.00	72.11	A
ATOM	967	O	ALA	215	-7.020	-3.161	-25.069	1.00	72.74	A
ATOM	968	N	LYS	216	-5.153	-2.076	-24.282	1.00	70.17	A
ATOM	969	H	LYS	216	-4.747	-1.165	-24.199	1.00	15.00	A
ATOM	970	CA	LYS	216	-4.482	-3.256	-23.626	1.00	67.38	A
ATOM	971	CB	LYS	216	-3.458	-2.691	-22.648	1.00	65.30	A
ATOM	972	CG	LYS	216	-2.217	-2.107	-23.321	1.00	66.86	A
ATOM	973	CD	LYS	216	-1.419	-3.149	-24.134	1.00	68.81	A
ATOM	974	CE	LYS	216	-0.082	-2.674	-24.740	1.00	67.51	A
ATOM	975	NZ	LYS	216	0.483	-3.722	-25.598	1.00	67.80	A
ATOM	976	HZ1	LYS	216	0.620	-4.590	-25.041	1.00	15.00	A
ATOM	977	HZ2	LYS	216	-0.168	-3.914	-26.385	1.00	15.00	A
ATOM	978	HZ3	LYS	216	1.401	-3.406	-25.973	1.00	15.00	A
ATOM	979	C	LYS	216	-5.321	-4.441	-22.993	1.00	66.99	A
ATOM	980	O	LYS	216	-6.462	-4.266	-22.575	1.00	69.90	A
ATOM	981	N	PRO	217	-4.835	-5.724	-22.952	1.00	65.06	A
ATOM	982	CD	PRO	217	-3.525	-6.262	-23.308	1.00	67.91	A
ATOM	983	CA	PRO	217	-5.792	-6.827	-22.626	1.00	62.80	A
ATOM	984	CB	PRO	217	-5.285	-8.004	-23.464	1.00	64.33	A
ATOM	985	CG	PRO	217	-3.755	-7.799	-23.338	1.00	69.63	A
ATOM	986	C	PRO	217	-5.837	-7.237	-21.150	1.00	59.77	A
ATOM	987	O	PRO	217	-4.747	-7.318	-20.589	1.00	58.81	A
ATOM	988	N	CYS	218	-7.115	-7.516	-20.627	1.00	55.45	A
ATOM	989	H	CYS	218	-7.874	-7.287	-21.233	1.00	15.00	A
ATOM	990	CA	CYS	218	-7.433	-7.929	-19.210	1.00	46.55	A
ATOM	991	CB	CYS	218	-8.105	-9.289	-19.079	1.00	44.69	A
ATOM	992	SG	CYS	218	-8.855	-9.822	-17.460	1.00	43.11	A
ATOM	993	C	CYS	218	-6.265	-7.994	-18.263	1.00	43.24	A
ATOM	994	O	CYS	218	-5.720	-9.026	-17.959	1.00	44.68	A
ATOM	995	N	GLY	219	-5.853	-6.820	-17.876	1.00	40.28	A
ATOM	996	H	GLY	219	-6.328	-5.961	-18.059	1.00	15.00	A
ATOM	997	CA	GLY	219	-4.659	-6.828	-17.070	1.00	36.27	A
ATOM	998	C	GLY	219	-5.017	-7.080	-15.643	1.00	33.86	A
ATOM	999	O	GLY	219	-5.906	-6.452	-15.097	1.00	34.90	A
ATOM	1000	N	GLN	220	-4.313	-7.996	-15.023	1.00	33.15	A
ATOM	1001	H	GLN	220	-3.835	-8.684	-15.580	1.00	15.00	A
ATOM	1002	CA	GLN	220	-4.448	-7.929	-13.578	1.00	29.92	A
ATOM	1003	CB	GLN	220	-4.298	-9.282	-12.936	1.00	27.81	A
ATOM	1004	CG	GLN	220	-5.380	-9.340	-11.883	1.00	30.94	A
ATOM	1005	CD	GLN	220	-5.285	-10.631	-11.132	1.00	36.37	A
ATOM	1006	OE1	GLN	220	-4.216	-10.969	-10.661	1.00	38.47	A
ATOM	1007	NE2	GLN	220	-6.425	-11.296	-10.977	1.00	37.61	A
ATOM	1008	HE21	GLN	220	-6.295	-12.235	-10.667	1.00	15.00	A
ATOM	1009	HE22	GLN	220	-7.373	-11.036	-11.200	1.00	15.00	A
ATOM	1010	C	GLN	220	-3.666	-6.845	-12.859	1.00	27.48	A
ATOM	1011	O	GLN	220	-2.461	-6.694	-12.999	1.00	27.61	A
ATOM	1012	N	GLN	221	-4.438	-6.040	-12.110	1.00	25.10	A
ATOM	1013	H	GLN	221	-5.433	-6.174	-12.143	1.00	15.00	A
ATOM	1014	CA	GLN	221	-3.803	-4.929	-11.387	1.00	22.41	A
ATOM	1015	CB	GLN	221	-4.077	-3.528	-11.949	1.00	22.12	A
ATOM	1016	CG	GLN	221	-3.284	-3.029	-13.163	1.00	32.16	A
ATOM	1017	CD	GLN	221	-3.795	-1.637	-13.405	1.00	34.69	A
ATOM	1018	OE1	GLN	221	-3.746	-0.763	-12.558	1.00	42.12	A
ATOM	1019	NE2	GLN	221	-4.648	-1.507	-14.398	1.00	34.93	A

FIGURE 2R

ATOM	1020	HE21	GLN	221	-4.981	-2.187	-15.042	1.00	15.00	A
ATOM	1021	HE22	GLN	221	-4.844	-0.551	-14.575	1.00	15.00	A
ATOM	1022	C	GLN	221	-4.227	-4.913	-9.948	1.00	19.54	A
ATOM	1023	O	GLN	221	-5.300	-5.381	-9.611	1.00	19.46	A
ATOM	1024	N	SER	222	-3.374	-4.330	-9.123	1.00	18.12	A
ATOM	1025	H	SER	222	-2.442	-4.098	-9.441	1.00	15.00	A
ATOM	1026	CA	SER	222	-3.851	-4.120	-7.752	1.00	19.45	A
ATOM	1027	CB	SER	222	-3.104	-4.947	-6.691	1.00	19.99	A
ATOM	1028	OG	SER	222	-3.096	-6.339	-7.053	1.00	24.64	A
ATOM	1029	HG	SER	222	-2.651	-6.336	-7.904	1.00	15.00	A
ATOM	1030	C	SER	222	-3.731	-2.688	-7.330	1.00	24.09	A
ATOM	1031	O	SER	222	-2.992	-1.929	-7.944	1.00	29.41	A
ATOM	1032	N	ILE	223	-4.534	-2.386	-6.283	1.00	22.81	A
ATOM	1033	H	ILE	223	-5.172	-3.127	-6.074	1.00	15.00	A
ATOM	1034	CA	ILE	223	-4.567	-1.122	-5.530	1.00	21.06	A
ATOM	1035	CB	ILE	223	-5.970	-0.490	-5.852	1.00	19.87	A
ATOM	1036	CG2	ILE	223	-6.564	0.315	-4.673	1.00	16.59	A
ATOM	1037	CG1	ILE	223	-5.911	0.278	-7.188	1.00	15.22	A
ATOM	1038	CD1	ILE	223	-7.229	0.868	-7.709	1.00	20.54	A
ATOM	1039	C	ILE	223	-4.367	-1.446	-4.007	1.00	21.62	A
ATOM	1040	O	ILE	223	-5.098	-2.269	-3.444	1.00	19.58	A
ATOM	1041	N	HIS	224	-3.429	-0.767	-3.340	1.00	19.73	A
ATOM	1042	H	HIS	224	-2.794	-0.230	-3.899	1.00	15.00	A
ATOM	1043	CA	HIS	224	-3.497	-0.671	-1.858	1.00	16.45	A
ATOM	1044	CB	HIS	224	-2.164	-1.183	-1.227	1.00	18.74	A
ATOM	1045	CG	HIS	224	-2.182	-1.442	0.296	1.00	14.92	A
ATOM	1046	ND1	HIS	224	-2.479	-2.628	0.882	1.00	15.33	A
ATOM	1047	HD1	HIS	224	-2.667	-3.515	0.505	1.00	15.00	A
ATOM	1048	CD2	HIS	224	-1.964	-0.524	1.310	1.00	13.79	A
ATOM	1049	NE2	HIS	224	-2.137	-1.127	2.517	1.00	10.52	A
ATOM	1050	CE1	HIS	224	-2.458	-2.411	2.232	1.00	11.70	A
ATOM	1051	C	HIS	224	-3.914	0.699	-1.284	1.00	15.18	A
ATOM	1052	O	HIS	224	-3.338	1.732	-1.520	1.00	14.36	A
ATOM	1053	N	LEU	225	-4.970	0.673	-0.468	1.00	16.85	A
ATOM	1054	H	LEU	225	-5.317	-0.238	-0.252	1.00	15.00	A
ATOM	1055	CA	LEU	225	-5.395	1.885	0.256	1.00	15.55	A
ATOM	1056	CB	LEU	225	-6.927	2.082	0.208	1.00	17.15	A
ATOM	1057	CG	LEU	225	-7.495	2.456	-1.154	1.00	18.03	A
ATOM	1058	CD1	LEU	225	-6.792	3.659	-1.774	1.00	19.34	A
ATOM	1059	CD2	LEU	225	-8.994	2.659	-1.098	1.00	13.66	A
ATOM	1060	C	LEU	225	-5.074	1.758	1.739	1.00	14.77	A
ATOM	1061	O	LEU	225	-5.347	0.726	2.345	1.00	12.20	A
ATOM	1062	N	GLY	226	-4.544	2.829	2.344	1.00	18.04	A
ATOM	1063	H	GLY	226	-4.218	3.616	1.813	1.00	15.00	A
ATOM	1064	CA	GLY	226	-4.541	2.833	3.841	1.00	18.37	A
ATOM	1065	C	GLY	226	-4.193	4.171	4.544	1.00	17.08	A
ATOM	1066	O	GLY	226	-3.389	4.906	4.055	1.00	13.75	A
ATOM	1067	N	GLY	227	-4.781	4.457	5.725	1.00	16.30	A
ATOM	1068	H	GLY	227	-5.434	3.771	6.036	1.00	15.00	A
ATOM	1069	CA	GLY	227	-4.379	5.649	6.490	1.00	8.52	A
ATOM	1070	C	GLY	227	-4.935	5.631	7.959	1.00	12.75	A
ATOM	1071	O	GLY	227	-5.651	4.748	8.466	1.00	10.57	A
ATOM	1072	N	VAL	228	-4.588	6.698	8.675	1.00	9.23	A
ATOM	1073	H	VAL	228	-4.040	7.398	8.222	1.00	15.00	A
ATOM	1074	CA	VAL	228	-5.110	6.818	10.067	1.00	11.74	A
ATOM	1075	CB	VAL	228	-4.085	7.320	11.144	1.00	14.30	A
ATOM	1076	CG1	VAL	228	-2.830	6.445	11.333	1.00	10.73	A
ATOM	1077	CG2	VAL	228	-4.789	7.565	12.479	1.00	17.07	A
ATOM	1078	C	VAL	228	-6.238	7.803	10.098	1.00	9.03	A
ATOM	1079	O	VAL	228	-6.089	8.937	9.649	1.00	12.01	A

FIGURE 2S

ATOM	1080	N	PHE	229	-7.347	7.299	10.640	1.00	9.88	A
ATOM	1081	H	PHE	229	-7.329	6.332	10.922	1.00	15.00	A
ATOM	1082	CA	PHE	229	-8.566	8.106	10.772	1.00	11.18	A
ATOM	1083	CB	PHE	229	-9.578	7.687	9.686	1.00	8.01	A
ATOM	1084	CG	PHE	229	-9.063	7.912	8.233	1.00	8.40	A
ATOM	1085	CD1	PHE	229	-9.140	9.196	7.649	1.00	10.03	A
ATOM	1086	CD2	PHE	229	-8.433	6.883	7.517	1.00	6.57	A
ATOM	1087	CE1	PHE	229	-8.512	9.443	6.395	1.00	5.18	A
ATOM	1088	CE2	PHE	229	-7.771	7.128	6.282	1.00	4.26	A
ATOM	1089	CZ	PHE	229	-7.813	8.424	5.731	1.00	5.71	A
ATOM	1090	C	PHE	229	-9.202	8.014	12.197	1.00	14.39	A
ATOM	1091	O	PHE	229	-9.116	7.000	12.870	1.00	13.92	A
ATOM	1092	N	GLU	230	-9.863	9.064	12.672	1.00	17.93	A
ATOM	1093	H	GLU	230	-9.912	9.892	12.113	1.00	15.00	A
ATOM	1094	CA	GLU	230	-10.856	8.944	13.770	1.00	18.08	A
ATOM	1095	CB	GLU	230	-11.218	10.303	14.393	1.00	16.17	A
ATOM	1096	CG	GLU	230	-11.068	10.090	15.889	1.00	27.69	A
ATOM	1097	CD	GLU	230	-12.314	10.091	16.805	1.00	33.06	A
ATOM	1098	OE1	GLU	230	-13.355	10.707	16.552	1.00	38.26	A
ATOM	1099	OE2	GLU	230	-12.218	9.477	17.863	1.00	38.14	A
ATOM	1100	C	GLU	230	-12.225	8.268	13.453	1.00	18.70	A
ATOM	1101	O	GLU	230	-12.967	8.519	12.492	1.00	21.58	A
ATOM	1102	N	LEU	231	-12.542	7.334	14.361	1.00	13.79	A
ATOM	1103	H	LEU	231	-11.840	7.125	15.015	1.00	15.00	A
ATOM	1104	CA	LEU	231	-13.885	6.836	14.330	1.00	13.52	A
ATOM	1105	CB	LEU	231	-13.954	5.378	14.002	1.00	13.90	A
ATOM	1106	CG	LEU	231	-13.199	5.064	12.725	1.00	15.44	A
ATOM	1107	CD1	LEU	231	-13.781	5.712	11.436	1.00	10.24	A
ATOM	1108	CD2	LEU	231	-12.970	3.569	12.769	1.00	11.74	A
ATOM	1109	C	LEU	231	-14.638	7.074	15.591	1.00	14.88	A
ATOM	1110	O	LEU	231	-14.145	6.912	16.692	1.00	12.46	A
ATOM	1111	N	GLN	232	-15.891	7.411	15.350	1.00	19.40	A
ATOM	1112	H	GLN	232	-16.107	7.560	14.394	1.00	15.00	A
ATOM	1113	CA	GLN	232	-16.920	7.509	16.389	1.00	21.07	A
ATOM	1114	CB	GLN	232	-18.132	8.234	15.804	1.00	23.55	A
ATOM	1115	CG	GLN	232	-17.792	9.709	15.687	1.00	28.60	A
ATOM	1116	CD	GLN	232	-17.625	10.200	17.102	1.00	33.66	A
ATOM	1117	OE1	GLN	232	-18.623	10.472	17.742	1.00	38.08	A
ATOM	1118	NE2	GLN	232	-16.380	10.254	17.596	1.00	33.41	A
ATOM	1119	HE21	GLN	232	-15.596	10.186	16.972	1.00	15.00	A
ATOM	1120	HE22	GLN	232	-16.387	10.470	18.576	1.00	15.00	A
ATOM	1121	C	GLN	232	-17.402	6.148	16.851	1.00	21.86	A
ATOM	1122	O	GLN	232	-17.368	5.218	16.052	1.00	21.58	A
ATOM	1123	N	PRO	233	-17.906	6.013	18.115	1.00	22.31	A
ATOM	1124	CD	PRO	233	-17.962	7.033	19.168	1.00	21.41	A
ATOM	1125	CA	PRO	233	-18.570	4.747	18.442	1.00	21.21	A
ATOM	1126	CB	PRO	233	-19.013	4.987	19.866	1.00	23.88	A
ATOM	1127	CG	PRO	233	-18.661	6.404	20.339	1.00	20.95	A
ATOM	1128	C	PRO	233	-19.667	4.417	17.434	1.00	23.66	A
ATOM	1129	O	PRO	233	-20.275	5.319	16.875	1.00	26.89	A
ATOM	1130	N	GLY	234	-19.731	3.140	17.059	1.00	22.77	A
ATOM	1131	H	GLY	234	-19.082	2.466	17.417	1.00	15.00	A
ATOM	1132	CA	GLY	234	-20.766	2.767	16.072	1.00	19.45	A
ATOM	1133	C	GLY	234	-20.545	3.241	14.625	1.00	19.67	A
ATOM	1134	O	GLY	234	-21.299	2.980	13.715	1.00	23.81	A
ATOM	1135	N	ALA	235	-19.405	3.926	14.368	1.00	18.89	A
ATOM	1136	H	ALA	235	-19.096	4.485	15.135	1.00	15.00	A
ATOM	1137	CA	ALA	235	-18.431	3.515	13.296	1.00	22.17	A
ATOM	1138	CB	ALA	235	-18.193	2.042	13.039	1.00	6.68	A
ATOM	1139	C	ALA	235	-18.540	4.160	11.993	1.00	21.96	A

FIGURE 2T

ATOM	1140	C	ALA	235	-18.486	5.385	12.100	1.00	26.42	A
ATOM	1141	N	SER	236	-18.699	3.498	10.787	1.00	20.94	A
ATOM	1142	H	SER	236	-18.824	4.326	10.254	1.00	15.00	A
ATOM	1143	CA	SER	236	-18.630	2.227	9.961	1.00	17.60	A
ATOM	1144	CB	SER	236	-19.905	1.876	9.160	1.00	14.98	A
ATOM	1145	OG	SER	236	-20.662	0.908	9.833	1.00	21.35	A
ATOM	1146	HG	SER	236	-21.599	0.910	9.647	1.00	15.00	A
ATOM	1147	C	SER	236	-17.794	2.538	8.714	1.00	13.65	A
ATOM	1148	O	SER	236	-17.939	3.614	8.131	1.00	16.29	A
ATOM	1149	N	VAL	237	-16.986	1.567	8.286	1.00	14.95	A
ATOM	1150	H	VAL	237	-16.764	0.823	8.949	1.00	15.00	A
ATOM	1151	CA	VAL	237	-16.201	1.802	7.077	1.00	11.42	A
ATOM	1152	CB	VAL	237	-14.681	2.004	7.284	1.00	12.49	A
ATOM	1153	CG1	VAL	237	-14.113	0.726	7.939	1.00	13.10	A
ATOM	1154	CG2	VAL	237	-14.254	3.396	7.846	1.00	10.27	A
ATOM	1155	C	VAL	237	-16.468	0.746	6.035	1.00	8.76	A
ATOM	1156	O	VAL	237	-16.827	-0.363	6.341	1.00	12.84	A
ATOM	1157	N	PHE	238	-16.354	1.158	4.773	1.00	12.45	A
ATOM	1158	H	PHE	238	-16.139	2.128	4.652	1.00	15.00	A
ATOM	1159	CA	PHE	238	-16.521	0.213	3.653	1.00	11.21	A
ATOM	1160	CB	PHE	238	-18.013	0.137	3.322	1.00	13.00	A
ATOM	1161	CG	PHE	238	-18.634	1.468	2.899	1.00	12.17	A
ATOM	1162	CD1	PHE	238	-18.763	1.812	1.518	1.00	12.94	A
ATOM	1163	CD2	PHE	238	-19.135	2.332	3.887	1.00	10.55	A
ATOM	1164	CE1	PHE	238	-19.407	3.010	1.092	1.00	14.01	A
ATOM	1165	CE2	PHE	238	-19.786	3.504	3.470	1.00	12.74	A
ATOM	1166	CZ	PHE	238	-19.917	3.836	2.100	1.00	13.17	A
ATOM	1167	C	PHE	238	-15.725	0.582	2.379	1.00	11.20	A
ATOM	1168	O	PHE	238	-15.137	1.638	2.267	1.00	8.73	A
ATOM	1169	N	VAL	239	-15.726	-0.300	1.383	1.00	14.34	A
ATOM	1170	H	VAL	239	-16.187	-1.170	1.523	1.00	15.00	A
ATOM	1171	CA	VAL	239	-14.982	0.027	0.154	1.00	14.65	A
ATOM	1172	CB	VAL	239	-13.900	-1.043	-0.162	1.00	14.09	A
ATOM	1173	CG1	VAL	239	-13.004	-1.318	1.038	1.00	14.55	A
ATOM	1174	CG2	VAL	239	-13.064	-0.594	-1.361	1.00	14.74	A
ATOM	1175	C	VAL	239	-15.930	0.081	-1.043	1.00	18.32	A
ATOM	1176	O	VAL	239	-16.558	-0.903	-1.369	1.00	18.99	A
ATOM	1177	N	ASN	240	-16.000	1.207	-1.707	1.00	19.26	A
ATOM	1178	H	ASN	240	-15.420	1.947	-1.383	1.00	15.00	A
ATOM	1179	CA	ASN	240	-16.613	1.355	-3.031	1.00	21.66	A
ATOM	1180	CB	ASN	240	-16.850	2.856	-3.095	1.00	24.58	A
ATOM	1181	CG	ASN	240	-18.167	3.077	-3.708	1.00	29.09	A
ATOM	1182	OD1	ASN	240	-18.948	2.123	-3.740	1.00	35.44	A
ATOM	1183	ND2	ASN	240	-18.293	4.331	-4.166	1.00	34.71	A
ATOM	1184	HD21	ASN	240	-19.149	4.489	-4.657	1.00	15.00	A
ATOM	1185	C	ASN	240	-15.669	0.950	-4.184	1.00	20.96	A
ATOM	1186	O	ASN	240	-14.473	1.128	-4.058	1.00	20.99	A
ATOM	1187	N	VAL	241	-16.189	0.383	-5.275	1.00	21.52	A
ATOM	1188	H	VAL	241	-17.182	0.230	-5.295	1.00	15.00	A
ATOM	1189	CA	VAL	241	-15.387	0.439	-6.516	1.00	20.56	A
ATOM	1190	CB	VAL	241	-14.581	-0.850	-6.849	1.00	18.02	A
ATOM	1191	CG1	VAL	241	-15.501	-2.058	-7.063	1.00	15.06	A
ATOM	1192	CG2	VAL	241	-13.597	-1.259	-5.764	1.00	20.05	A
ATOM	1193	C	VAL	241	-16.253	0.758	-7.741	1.00	18.88	A
ATOM	1194	O	VAL	241	-17.441	0.500	-7.819	1.00	18.63	A
ATOM	1195	N	THR	242	-15.541	1.162	-8.762	1.00	21.24	A
ATOM	1196	H	THR	242	-14.704	1.653	-8.486	1.00	15.00	A
ATOM	1197	CA	THR	242	-16.246	1.476	-10.031	1.00	20.63	A
ATOM	1198	CB	THR	242	-15.342	2.269	-10.981	1.00	15.80	A
ATOM	1199	OG1	THR	242	-14.035	1.663	-10.953	1.00	17.72	A

FIGURE 2U

ATOM	1200	HG1	THR	242	-13.721	1.969	-11.812	1.00	15.00	A
ATOM	1201	CG2	THR	242	-15.238	3.732	-10.650	1.00	15.04	A
ATOM	1202	C	THR	242	-16.755	0.240	-10.783	1.00	18.92	A
ATOM	1203	O	THR	242	-17.846	0.198	-11.297	1.00	21.26	A
ATOM	1204	N	ASP	243	-15.923	-0.806	-10.718	1.00	20.98	A
ATOM	1205	H	ASP	243	-15.087	-0.580	-10.221	1.00	15.00	A
ATOM	1206	CA	ASP	243	-16.092	-1.977	-11.628	1.00	21.28	A
ATOM	1207	CB	ASP	243	-14.905	-2.126	-12.594	1.00	22.05	A
ATOM	1208	CG	ASP	243	-14.932	-0.954	-13.492	1.00	28.23	A
ATOM	1209	OD1	ASP	243	-14.314	0.051	-13.115	1.00	28.43	A
ATOM	1210	OD2	ASP	243	-15.588	-1.033	-14.535	1.00	33.00	A
ATOM	1211	C	ASP	243	-16.123	-3.308	-10.923	1.00	20.38	A
ATOM	1212	O	ASP	243	-15.148	-4.072	-10.967	1.00	20.43	A
ATOM	1213	N	PRO	244	-17.204	-3.553	-10.154	1.00	19.92	A
ATOM	1214	CD	PRO	244	-18.481	-2.871	-10.071	1.00	16.83	A
ATOM	1215	CA	PRO	244	-17.120	-4.706	-9.269	1.00	19.13	A
ATOM	1216	CB	PRO	244	-18.293	-4.535	-8.275	1.00	15.33	A
ATOM	1217	CG	PRO	244	-18.890	-3.174	-8.634	1.00	15.21	A
ATOM	1218	C	PRO	244	-16.975	-6.034	-9.974	1.00	19.29	A
ATOM	1219	O	PRO	244	-16.194	-6.859	-9.548	1.00	23.48	A
ATOM	1220	N	SER	245	-17.581	-6.163	-11.150	1.00	22.60	A
ATOM	1221	H	SER	245	-18.220	-5.459	-11.473	1.00	15.00	A
ATOM	1222	CA	SER	245	-17.414	-7.429	-11.942	1.00	25.50	A
ATOM	1223	CB	SER	245	-18.256	-7.369	-13.234	1.00	21.36	A
ATOM	1224	OG	SER	245	-19.667	-7.567	-12.981	1.00	38.26	A
ATOM	1225	HG	SER	245	-19.848	-7.390	-12.038	1.00	15.00	A
ATOM	1226	C	SER	245	-15.955	-7.776	-12.328	1.00	24.14	A
ATOM	1227	O	SER	245	-15.477	-8.859	-12.623	1.00	24.84	A
ATOM	1228	N	GLN	246	-15.177	-6.689	-12.385	1.00	28.52	A
ATOM	1229	H	GLN	246	-15.638	-5.804	-12.265	1.00	15.00	A
ATOM	1230	CA	GLN	246	-13.743	-6.923	-12.590	1.00	26.45	A
ATOM	1231	CB	GLN	246	-13.144	-5.645	-13.233	1.00	29.90	A
ATOM	1232	CG	GLN	246	-13.403	-5.435	-14.758	1.00	26.84	A
ATOM	1233	CD	GLN	246	-14.862	-5.341	-15.129	1.00	21.60	A
ATOM	1234	OE1	GLN	246	-15.538	-4.503	-14.616	1.00	24.20	A
ATOM	1235	NE2	GLN	246	-15.334	-6.234	-15.975	1.00	26.15	A
ATOM	1236	HE21	GLN	246	-14.763	-6.924	-16.423	1.00	15.00	A
ATOM	1237	HE22	GLN	246	-16.320	-6.119	-16.084	1.00	15.00	A
ATOM	1238	C	GLN	246	-12.936	-7.372	-11.363	1.00	27.14	A
ATOM	1239	O	GLN	246	-11.721	-7.570	-11.454	1.00	25.73	A
ATOM	1240	N	VAL	247	-13.615	-7.395	-10.196	1.00	23.70	A
ATOM	1241	H	VAL	247	-14.600	-7.594	-10.146	1.00	15.00	A
ATOM	1242	CA	VAL	247	-12.728	-7.569	-9.097	1.00	21.91	A
ATOM	1243	CB	VAL	247	-13.156	-6.814	-7.859	1.00	21.59	A
ATOM	1244	CG1	VAL	247	-14.027	-7.616	-6.962	1.00	24.52	A
ATOM	1245	CG2	VAL	247	-13.680	-5.409	-8.167	1.00	21.61	A
ATOM	1246	C	VAL	247	-12.258	-8.998	-8.910	1.00	21.55	A
ATOM	1247	O	VAL	247	-12.946	-9.912	-9.251	1.00	19.53	A
ATOM	1248	N	SER	248	-11.000	-9.152	-8.444	1.00	21.31	A
ATOM	1249	H	SER	248	-10.558	-8.342	-8.070	1.00	15.00	A
ATOM	1250	CA	SER	248	-10.414	-10.499	-8.327	1.00	21.97	A
ATOM	1251	CB	SER	248	-8.939	-10.571	-8.828	1.00	23.61	A
ATOM	1252	OG	SER	248	-8.860	-9.952	-10.128	1.00	20.21	A
ATOM	1253	HG	SER	248	-9.752	-10.027	-10.496	1.00	15.00	A
ATOM	1254	C	SER	248	-10.538	-11.076	-6.946	1.00	19.28	A
ATOM	1255	O	SER	248	-10.048	-10.409	-6.052	1.00	20.64	A
ATOM	1256	N	HIS	249	-11.269	-12.204	-6.814	1.00	18.72	A
ATOM	1257	H	HIS	249	-11.284	-12.753	-7.674	1.00	15.00	A
ATOM	1258	CA	HIS	249	-11.640	-12.673	-5.478	1.00	17.22	A
ATOM	1259	CB	HIS	249	-13.080	-13.152	-5.484	1.00	13.10	A

FIGURE 2V

ATOM	1260	CG	HIS	249	-13.919	-11.905	-5.580	1.00	10.13	A
ATOM	1261	ND1	HIS	249	-14.137	-11.129	-4.486	1.00	13.47	A
ATOM	1262	HD1	HIS	249	-13.720	-11.294	-3.611	1.00	15.00	A
ATOM	1263	CD2	HIS	249	-14.662	-11.414	-6.610	1.00	10.62	A
ATOM	1264	NE2	HIS	249	-15.317	-10.347	-6.134	1.00	15.51	A
ATOM	1265	CE1	HIS	249	-15.018	-10.142	-4.821	1.00	12.36	A
ATOM	1266	C	HIS	249	-10.701	-13.683	-4.958	1.00	23.58	A
ATOM	1267	O	HIS	249	-11.103	-14.729	-4.359	1.00	21.98	A
ATOM	1268	N	GLY	250	-9.398	-13.258	-4.878	1.00	29.10	A
ATOM	1269	H	GLY	250	-9.252	-12.351	-5.253	1.00	15.00	A
ATOM	1270	CA	GLY	250	-8.410	-14.041	-4.115	1.00	24.27	A
ATOM	1271	C	GLY	250	-8.336	-15.372	-4.743	1.00	25.93	A
ATOM	1272	O	GLY	250	-8.940	-15.520	-5.795	1.00	29.26	A
ATOM	1273	N	THR	251	-7.594	-16.302	-4.127	1.00	22.38	A
ATOM	1274	H	THR	251	-7.485	-17.038	-4.804	1.00	15.00	A
ATOM	1275	CA	THR	251	-7.111	-16.139	-2.725	1.00	21.12	A
ATOM	1276	CB	THR	251	-6.988	-17.525	-1.933	1.00	24.76	A
ATOM	1277	OG1	THR	251	-5.877	-17.641	-0.981	1.00	22.90	A
ATOM	1278	HG1	THR	251	-6.063	-18.366	-0.381	1.00	15.00	A
ATOM	1279	CG2	THR	251	-6.968	-18.722	-2.890	1.00	22.77	A
ATOM	1280	C	THR	251	-5.952	-15.158	-2.473	1.00	17.96	A
ATOM	1281	O	THR	251	-4.969	-15.043	-3.213	1.00	12.30	A
ATOM	1282	N	GLY	252	-6.241	-14.367	-1.419	1.00	16.85	A
ATOM	1283	H	GLY	252	-7.093	-14.432	-0.862	1.00	15.00	A
ATOM	1284	CA	GLY	252	-5.277	-13.375	-0.928	1.00	13.16	A
ATOM	1285	C	GLY	252	-5.357	-12.058	-1.670	1.00	15.51	A
ATOM	1286	O	GLY	252	-4.580	-11.168	-1.439	1.00	15.18	A
ATOM	1287	N	PHE	253	-6.189	-12.063	-2.744	1.00	16.66	A
ATOM	1288	H	PHE	253	-6.868	-12.805	-2.761	1.00	15.00	A
ATOM	1289	CA	PHE	253	-6.110	-10.892	-3.651	1.00	15.77	A
ATOM	1290	CB	PHE	253	-6.649	-11.216	-5.100	1.00	17.11	A
ATOM	1291	CG	PHE	253	-5.595	-11.840	-5.994	1.00	11.82	A
ATOM	1292	CD1	PHE	253	-4.385	-11.175	-6.231	1.00	13.69	A
ATOM	1293	CD2	PHE	253	-5.845	-13.089	-6.558	1.00	18.59	A
ATOM	1294	CE1	PHE	253	-3.364	-11.771	-6.993	1.00	14.39	A
ATOM	1295	CE2	PHE	253	-4.840	-13.680	-7.363	1.00	21.37	A
ATOM	1296	CZ	PHE	253	-3.612	-13.014	-7.562	1.00	15.72	A
ATOM	1297	C	PHE	253	-6.740	-9.599	-3.147	1.00	13.88	A
ATOM	1298	O	PHE	253	-6.347	-8.477	-3.453	1.00	14.27	A
ATOM	1299	N	THR	254	-7.865	-9.837	-2.502	1.00	14.00	A
ATOM	1300	H	THR	254	-8.079	-10.748	-2.124	1.00	15.00	A
ATOM	1301	CA	THR	254	-8.741	-8.681	-2.185	1.00	14.09	A
ATOM	1302	CB	THR	254	-9.908	-8.469	-3.201	1.00	11.66	A
ATOM	1303	OG1	THR	254	-9.414	-8.325	-4.536	1.00	13.08	A
ATOM	1304	HG1	THR	254	-9.826	-9.054	-4.992	1.00	15.00	A
ATOM	1305	CG2	THR	254	-10.882	-7.321	-2.885	1.00	13.78	A
ATOM	1306	C	THR	254	-9.270	-8.779	-0.738	1.00	12.36	A
ATOM	1307	O	THR	254	-9.906	-9.695	-0.240	1.00	14.54	A
ATOM	1308	N	SER	255	-9.007	-7.683	-0.027	1.00	13.42	A
ATOM	1309	H	SER	255	-8.425	-7.021	-0.490	1.00	15.00	A
ATOM	1310	CA	SER	255	-9.032	-7.725	1.431	1.00	7.59	A
ATOM	1311	CB	SER	255	-7.793	-8.466	1.976	1.00	6.39	A
ATOM	1312	OG	SER	255	-6.704	-7.560	2.041	1.00	9.69	A
ATOM	1313	HG	SER	255	-5.920	-8.031	1.741	1.00	15.00	A
ATOM	1314	C	SER	255	-9.248	-6.341	2.085	1.00	10.05	A
ATOM	1315	O	SER	255	-9.191	-5.254	1.492	1.00	15.21	A
ATOM	1316	N	PHE	256	-9.653	-6.385	3.369	1.00	8.54	A
ATOM	1317	H	PHE	256	-9.700	-7.323	3.733	1.00	15.00	A
ATOM	1318	CA	PHE	256	-10.114	-5.168	4.035	1.00	7.94	A
ATOM	1319	CB	PHE	256	-11.605	-5.009	3.679	1.00	11.65	A

FIGURE 2W

ATOM	1320	CG	PHE	256	-12.376	-3.824	4.235	1.00	8.72	A
ATOM	1321	CD1	PHE	256	-11.766	-2.570	4.533	1.00	11.20	A
ATOM	1322	CD2	PHE	256	-13.756	-3.976	4.327	1.00	6.12	A
ATOM	1323	CE1	PHE	256	-12.503	-1.490	5.034	1.00	11.49	A
ATOM	1324	CE2	PHE	256	-14.514	-2.849	4.734	1.00	6.86	A
ATOM	1325	CZ	PHE	256	-13.862	-1.657	5.211	1.00	9.27	A
ATOM	1326	C	PHE	256	-9.933	-5.268	5.560	1.00	11.92	A
ATOM	1327	O	PHE	256	-10.195	-6.290	6.177	1.00	9.43	A
ATOM	1328	N	GLY	257	-9.420	-4.207	6.169	1.00	10.57	A
ATOM	1329	H	GLY	257	-9.217	-3.365	5.653	1.00	15.00	A
ATOM	1330	CA	GLY	257	-9.368	-4.406	7.612	1.00	11.26	A
ATOM	1331	C	GLY	257	-8.965	-3.122	8.287	1.00	11.14	A
ATOM	1332	O	GLY	257	-8.916	-2.068	7.679	1.00	10.81	A
ATOM	1333	N	LEU	258	-8.688	-3.277	9.565	1.00	12.61	A
ATOM	1334	H	LEU	258	-8.776	-4.204	9.943	1.00	15.00	A
ATOM	1335	CA	LEU	258	-8.434	-2.098	10.426	1.00	14.72	A
ATOM	1336	CB	LEU	258	-9.751	-1.212	10.704	1.00	14.67	A
ATOM	1337	CG	LEU	258	-10.991	-1.863	11.379	1.00	18.02	A
ATOM	1338	CD1	LEU	258	-12.317	-1.125	11.094	1.00	15.05	A
ATOM	1339	CD2	LEU	258	-10.743	-2.047	12.905	1.00	15.42	A
ATOM	1340	C	LEU	258	-7.737	-2.525	11.709	1.00	11.84	A
ATOM	1341	O	LEU	258	-7.851	-3.690	12.096	1.00	7.91	A
ATOM	1342	N	LEU	259	-7.058	-1.537	12.343	1.00	11.64	A
ATOM	1343	H	LEU	259	-6.883	-0.685	11.844	1.00	15.00	A
ATOM	1344	CA	LEU	259	-6.581	-1.780	13.714	1.00	9.53	A
ATOM	1345	CB	LEU	259	-5.155	-2.417	13.831	1.00	7.40	A
ATOM	1346	CG	LEU	259	-4.194	-1.621	12.931	1.00	11.40	A
ATOM	1347	CD1	LEU	259	-3.355	-2.412	11.926	1.00	7.83	A
ATOM	1348	CD2	LEU	259	-3.379	-0.670	13.808	1.00	13.30	A
ATOM	1349	C	LEU	259	-6.652	-0.497	14.531	1.00	10.40	A
ATOM	1350	O	LEU	259	-6.202	0.556	14.082	1.00	9.73	A
ATOM	1351	N	LYS	260	-7.193	-0.629	15.762	1.00	12.00	A
ATOM	1352	H	LYS	260	-7.395	-1.553	16.115	1.00	15.00	A
ATOM	1353	CA	LYS	260	-7.069	0.521	16.693	1.00	13.51	A
ATOM	1354	CB	LYS	260	-8.014	0.312	17.885	1.00	13.49	A
ATOM	1355	CG	LYS	260	-8.378	1.656	18.521	1.00	17.16	A
ATOM	1356	CD	LYS	260	-9.435	1.456	19.596	1.00	12.01	A
ATOM	1357	CE	LYS	260	-10.151	2.681	20.121	1.00	11.41	A
ATOM	1358	NZ	LYS	260	-9.175	3.595	20.697	1.00	13.33	A
ATOM	1359	HZ1	LYS	260	-8.534	3.932	19.954	1.00	15.00	A
ATOM	1360	HZ2	LYS	260	-9.693	4.404	21.095	1.00	15.00	A
ATOM	1361	HZ3	LYS	260	-8.638	3.136	21.458	1.00	15.00	A
ATOM	1362	C	LYS	260	-5.648	0.921	17.125	1.00	16.54	A
ATOM	1363	O	LYS	260	-4.828	0.112	17.481	1.00	15.61	A
ATOM	1364	N	LEU	261	-5.353	2.199	17.015	1.00	14.78	A
ATOM	1365	H	LEU	261	-6.089	2.838	16.856	1.00	15.00	A
ATOM	1366	CB	LEU	261	-3.705	4.005	17.185	1.00	19.53	A
ATOM	1367	CG	LEU	261	-3.177	4.309	15.787	1.00	16.82	A
ATOM	1368	CD1	LEU	261	-3.010	5.779	15.767	1.00	12.45	A
ATOM	1369	CD2	LEU	261	-4.010	3.906	14.577	1.00	18.20	A
ATOM	1370	C	LEU	261	-4.243	2.667	19.225	1.00	20.80	A
ATOM	1371	OCT1	LEU	261	-5.363	2.741	19.746	1.00	22.59	A
ATOM	1372	OCT2	LEU	261	-3.221	2.696	19.913	1.00	26.97	A
ATOM	1373	CA	LEU	261	-4.122	2.604	17.684	1.00	18.13	A
ATOM	1374	O	HOH	501	-20.040	9.837	7.596	1.00	16.33	W
ATOM	1375	H1	HOH	501	-19.411	10.547	7.803	1.00	10.00	W
ATOM	1376	H2	HOH	501	-19.615	9.317	6.900	1.00	10.00	W
ATOM	1377	O	HOH	502	-9.727	11.545	10.743	1.00	10.94	W
ATOM	1378	H1	HOH	502	-10.039	11.934	9.919	1.00	15.00	W
ATOM	1379	H2	HOH	502	-10.233	12.125	11.315	1.00	15.00	W

FIGURE 2X

ATOM	1380	O	HOH	503	-8.158	13.188	13.681	1.00	30.64	W
ATOM	1381	H1	HOH	503	-8.715	12.529	13.277	1.00	15.00	W
ATOM	1382	H2	HOH	503	-8.700	13.944	13.574	1.00	15.00	W
ATOM	1383	O	HOH	504	-16.772	8.440	12.789	1.00	12.00	W
ATOM	1384	H1	HOH	504	-17.194	9.259	12.886	1.00	10.00	W
ATOM	1385	H2	HOH	504	-15.921	8.763	12.582	1.00	10.00	W
ATOM	1386	O	HOH	505	-25.173	7.297	7.925	1.00	47.03	W
ATOM	1387	H1	HOH	505	-24.690	8.064	8.239	1.00	10.00	W
ATOM	1388	H2	HOH	505	-25.990	7.684	7.583	1.00	10.00	W
ATOM	1389	O	HOH	506	-23.612	14.948	13.859	1.00	36.14	W
ATOM	1390	H1	HOH	506	-24.160	15.702	13.605	1.00	10.00	W
ATOM	1391	H2	HOH	506	-23.282	15.191	14.748	1.00	10.00	W
ATOM	1392	O	HOH	507	-17.329	-8.460	-7.186	1.00	34.02	W
ATOM	1393	O	HOH	508	-18.687	-7.253	-3.843	1.00	63.14	W
ATOM	1394	O	HOH	509	-7.157	11.327	3.239	1.00	22.26	W
ATOM	1395	O	HOH	510	-19.322	7.486	-2.227	1.00	37.69	W
ATOM	1396	O	HOH	511	-14.645	-7.711	-1.931	1.00	26.48	W
ATOM	1397	O	HOH	512	-18.377	-9.754	12.556	1.00	24.86	W
ATOM	1398	O	HOH	513	0.030	0.048	-13.455	1.00	26.05	W
ATOM	1399	O	HOH	514	-8.938	5.945	22.862	1.00	34.39	W
ATOM	1400	O	HOH	515	-29.446	-4.922	-7.247	1.00	41.61	W
ATOM	1401	O	HOH	516	-12.982	10.220	10.038	1.00	47.16	W
ATOM	1402	O	HOH	517	-21.797	-9.377	7.242	1.00	60.65	W
ATOM	1403	O	HOH	518	-7.867	8.165	19.484	1.00	40.46	W
ATOM	1404	O	HOH	520	-15.588	-14.701	14.628	1.00	63.80	W
ATOM	1405	O	HOH	521	-21.844	7.778	20.415	1.00	35.72	W
ATOM	1406	O	HOH	522	-6.555	-3.308	-15.790	1.00	33.63	W
ATOM	1407	O	HOH	523	-9.046	-13.476	-8.051	1.00	44.08	W
ATOM	1408	O	HOH	524	-17.413	-9.311	17.071	1.00	34.06	W
ATOM	1409	O	HOH	525	-23.838	4.781	19.884	1.00	37.99	W
ATOM	1410	O	HOH	526	-26.323	15.525	10.379	1.00	72.49	W
ATOM	1411	O	HOH	527	-3.167	-13.749	-10.820	1.00	43.99	W
ATOM	1412	O	HOH	528	-0.470	2.513	17.943	1.00	63.68	W
ATOM	1413	O	HOH	529	-5.580	-12.778	-14.864	1.00	47.52	W
ATOM	1414	O	HOH	530	-2.641	7.004	2.495	1.00	18.07	W
ATOM	1415	O	HOH	531	-6.472	12.847	0.156	1.00	24.96	W
ATOM	1416	O	HOH	532	-10.363	-16.426	-0.360	1.00	63.56	W
ATOM	1417	O	HOH	533	-1.378	-17.183	-13.053	1.00	67.67	W
ATOM	1418	O	HOH	534	-4.774	9.073	-0.651	1.00	23.36	W
ATOM	1419	O	HOH	535	-18.917	-13.857	6.913	1.00	32.28	W
ATOM	1420	O	HOH	536	-23.062	3.270	0.454	1.00	52.03	W
ATOM	1421	O	HOH	537	-25.906	9.022	16.986	1.00	44.75	W
ATOM	1422	O	HOH	538	-21.729	16.972	17.027	1.00	53.12	W
ATOM	1423	O	HOH	539	-9.084	11.806	17.034	1.00	70.90	W
ATOM	1424	O	HOH	540	-10.938	-13.296	15.207	1.00	35.65	W
ATOM	1425	O	HOH	541	-6.068	13.255	17.989	1.00	67.36	W
ATOM	1426	O	HOH	542	-20.593	-11.039	-9.003	1.00	96.30	W
ATOM	1427	O	HOH	543	-15.926	13.397	1.269	1.00	35.72	W
ATOM	1428	O	HOH	544	-24.591	-7.285	-2.353	1.00	43.42	W
ATOM	1429	O	HOH	545	-25.859	-2.666	-15.747	1.00	53.56	W
ATOM	1430	O	HOH	546	-23.074	-1.533	11.026	1.00	56.44	W
ATOM	1431	O	HOH	548	-8.941	-12.649	-12.394	1.00	64.34	W
ATOM	1432	O	HOH	549	-14.150	6.038	-12.250	1.00	41.38	W
ATOM	1433	O	HOH	550	-14.274	-0.613	18.441	1.00	56.17	W
ATOM	1434	O	HOH	551	-12.241	-19.609	8.637	1.00	80.90	W
ATOM	1435	O	HOH	552	-10.316	15.578	10.166	1.00	39.58	W
ATOM	1436	O	HOH	553	-15.367	10.941	14.659	1.00	40.40	W
ATOM	1437	O	HOH	554	-2.322	1.830	-5.294	1.00	33.65	W
ATOM	1438	O	HOH	555	-22.393	-14.875	-4.217	1.00	52.40	W
ATOM	1439	O	HOH	556	-22.120	14.279	7.189	1.00	38.55	W

FIGURE 2Y

ATOM	1440	O	HOH	557	-28.833	6.135	9.560	1.00	37.40	W
ATOM	1441	O	HOH	558	-5.554	-16.509	13.192	1.00	88.88	W
ATOM	1442	O	HOH	559	-22.996	12.522	1.162	1.00	63.77	W
ATOM	1443	O	HOH	560	-13.764	2.268	-14.743	1.00	27.47	W
ATOM	1444	O	HOH	561	-15.556	7.750	-5.628	1.00	75.88	W
ATOM	1445	O	HOH	562	-1.970	-15.363	-17.719	1.00	76.30	W
ATOM	1446	O	HOH	563	-18.939	-0.335	-13.842	1.00	48.39	W
ATOM	1447	O	HOH	564	-12.619	14.760	-6.974	1.00	100.59	W
ATOM	1448	O	HOH	565	-9.491	18.046	13.682	1.00	87.45	W
ATOM	1449	O	HOH	566	-11.655	-11.140	22.481	1.00	28.88	W
ATOM	1450	O	HOH	567	-24.072	-3.264	-0.332	1.00	35.13	W
ATOM	1451	O	HOH	568	-27.455	0.119	-7.117	1.00	71.07	W
ATOM	1452	O	HOH	569	-14.604	3.516	-6.119	1.00	59.45	W
ATOM	1453	O	HOH	570	-2.635	-9.566	-16.973	1.00	59.09	W
ATOM	1454	O	HOH	571	-18.841	4.066	-7.543	1.00	34.10	W
ATOM	1455	O	HOH	572	-24.996	1.301	17.953	1.00	70.45	W
ATOM	1456	O	HOH	573	-14.666	16.471	8.995	1.00	62.77	W
ATOM	1457	O	HOH	574	-14.786	1.426	10.949	1.00	82.68	W
ATOM	1458	O	HOH	575	-16.584	-14.717	-4.352	1.00	29.09	W
ATOM	1459	O	HOH	576	-16.273	-4.590	6.109	1.00	104.64	W
ATOM	1460	O	HOH	577	-25.471	-0.127	-2.510	1.00	62.74	W
ATOM	1461	O	HOH	578	-7.334	-17.173	19.514	1.00	89.62	W
ATOM	1462	O	HOH	579	-21.060	14.259	19.996	1.00	69.59	W
ATOM	1463	O	HOH	580	-19.286	4.057	-12.816	1.00	60.37	W
ATOM	1464	O	HOH	581	-22.445	-15.840	0.317	1.00	58.24	W
ATOM	1465	O	HOH	582	-22.434	-10.539	12.489	1.00	70.25	W
ATOM	1466	O	HOH	583	-21.327	3.668	-2.500	1.00	39.32	W
ATOM	1467	O	HOH	584	-25.325	5.247	16.919	1.00	41.31	W
ATOM	1468	O	HOH	585	-24.945	-10.718	-2.375	1.00	38.85	W
ATOM	1469	O	HOH	586	-24.342	-13.003	1.927	1.00	70.58	W
ATOM	1470	O	HOH	587	-18.020	11.871	11.358	1.00	64.47	W
ATOM	1471	O	HOH	588	-27.135	6.965	13.151	1.00	53.96	W
ATOM	1472	O	HOH	589	-14.982	-16.230	-2.494	1.00	30.24	W
ATOM	1473	O	HOH	590	-5.646	14.418	-2.232	1.00	41.78	W
ATOM	1474	O	HOH	591	-2.745	-0.153	-17.104	1.00	55.19	W
ATOM	1475	O	HOH	592	-3.397	-7.012	22.477	1.00	59.46	W
ATOM	1476	O	HOH	593	-32.916	-4.705	-4.143	1.00	51.88	W
ATOM	1477	O	HOH	594	-10.913	-18.855	-3.503	1.00	42.29	W
ATOM	1478	O	HOH	595	-24.157	1.821	-6.165	1.00	47.43	W
END										

FIGURE 3A

FIGURE 3B

FIGURE 4A

FIGURE 4B

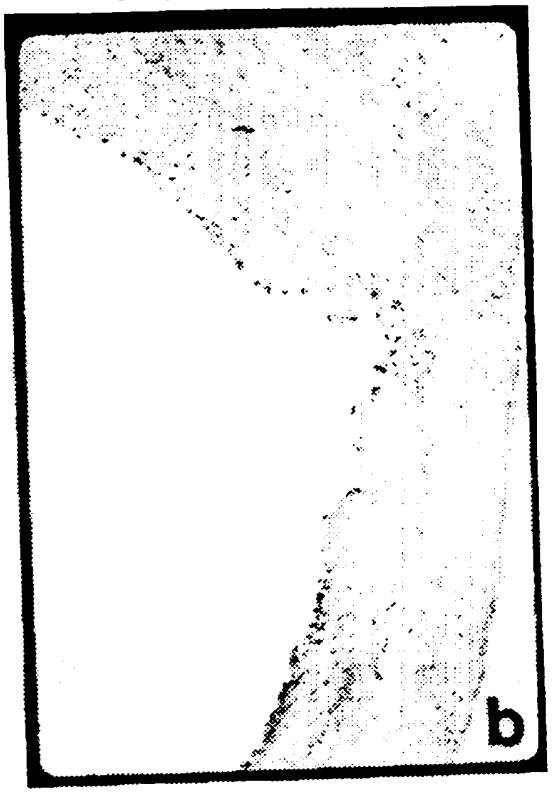

FIGURE 5A

FIGURE 5B

FIGURE 6A

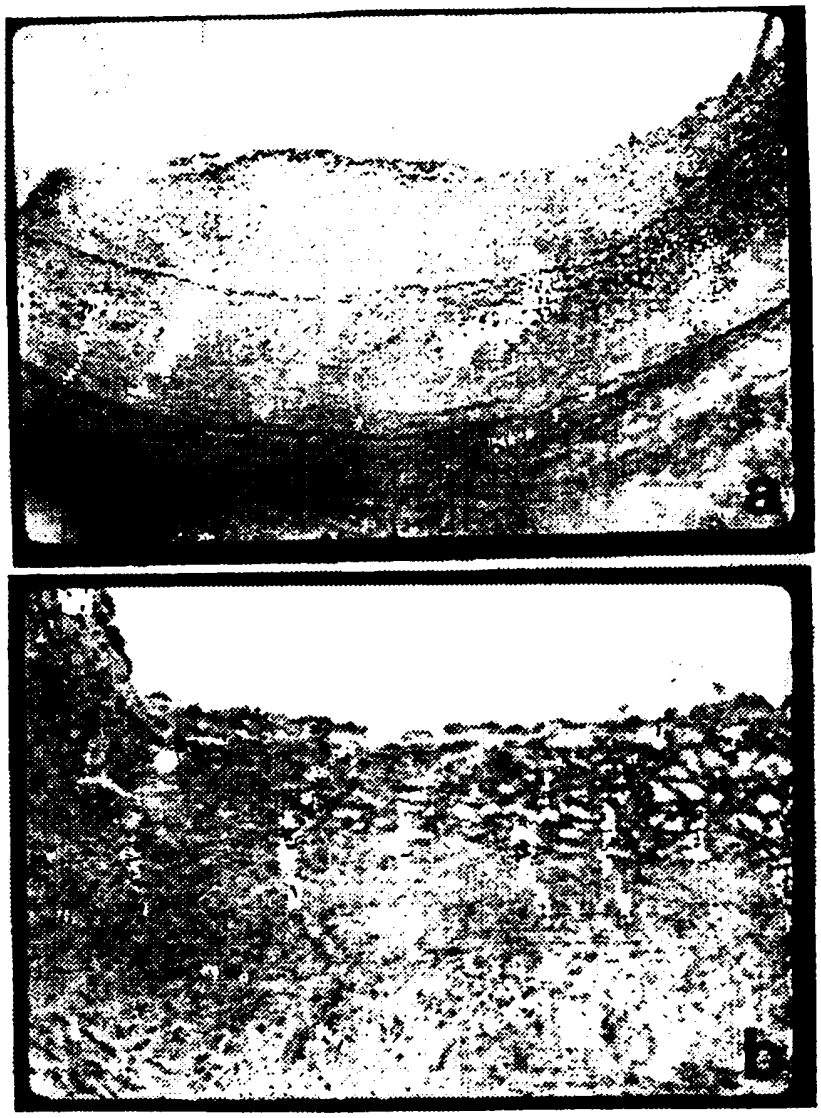

FIGURE 6B

FIGURE 6C

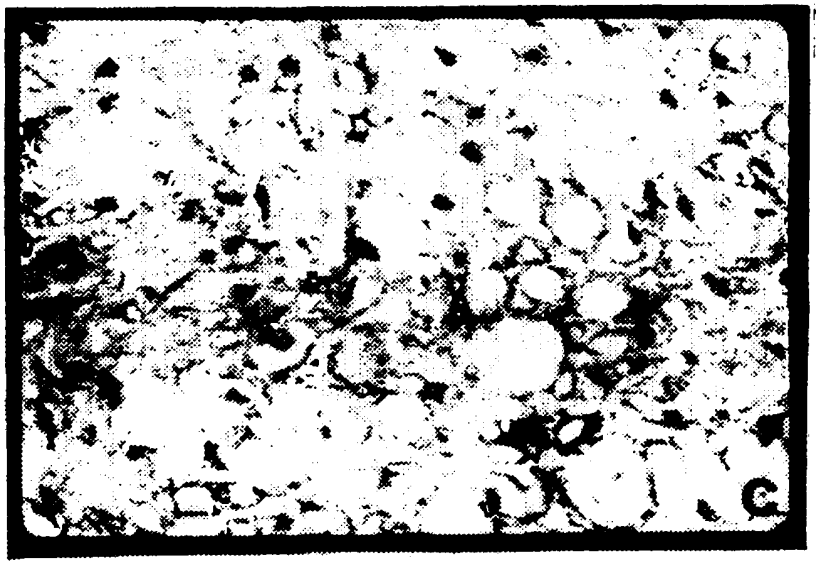

FIGURE 7A

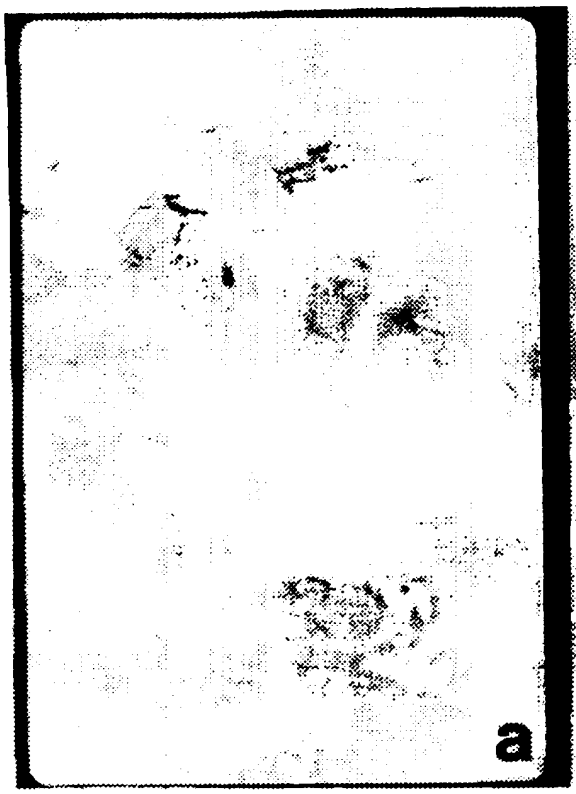

FIGURE 7B

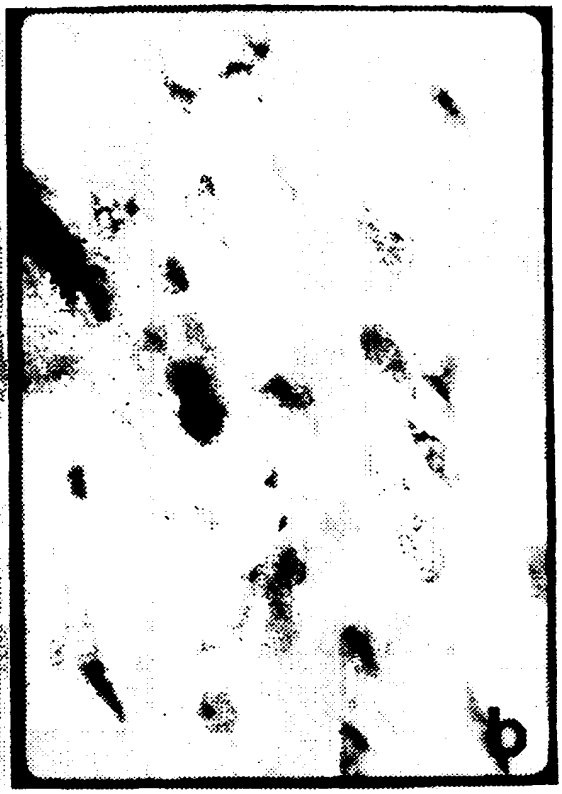

FIGURE 7C

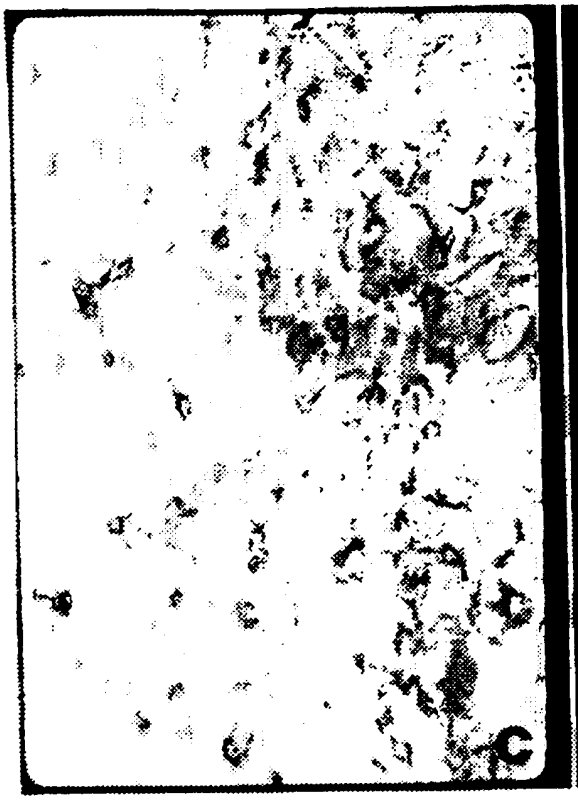

FIGURE 7D

FIGURE 8A

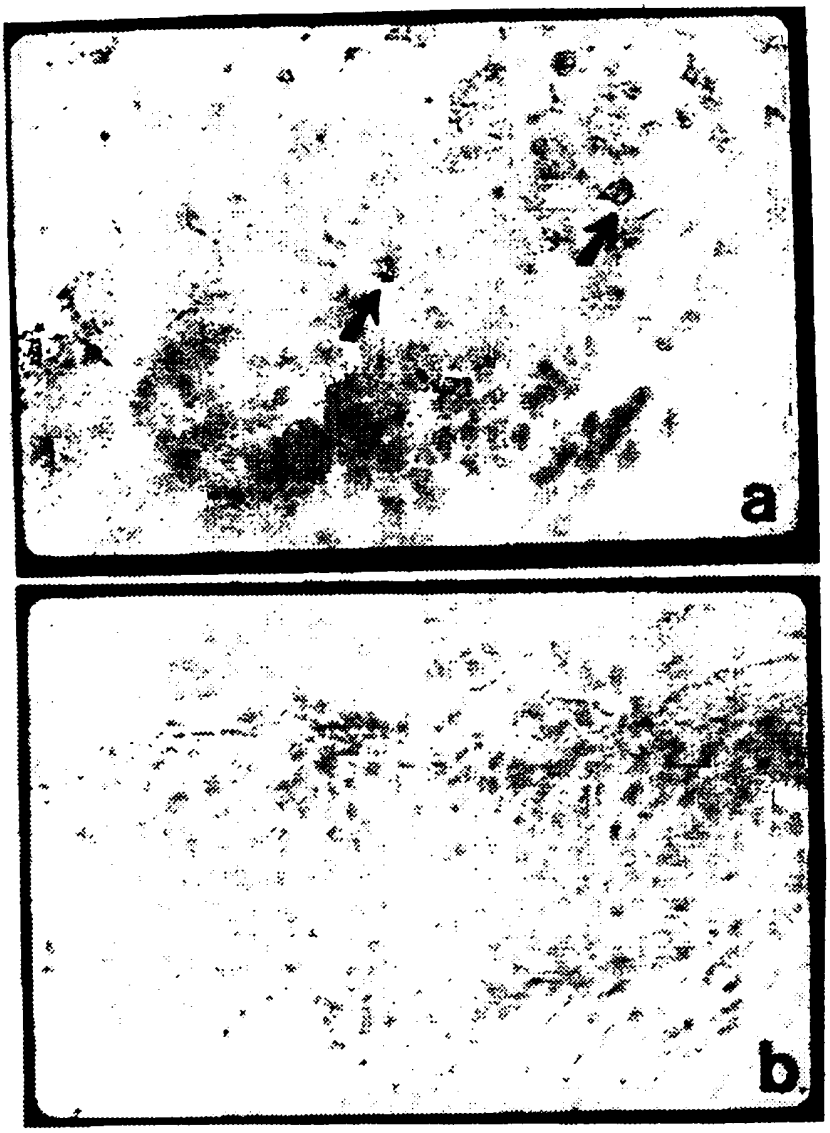

FIGURE 8B

FIGURE 8C

FIGURE 9A

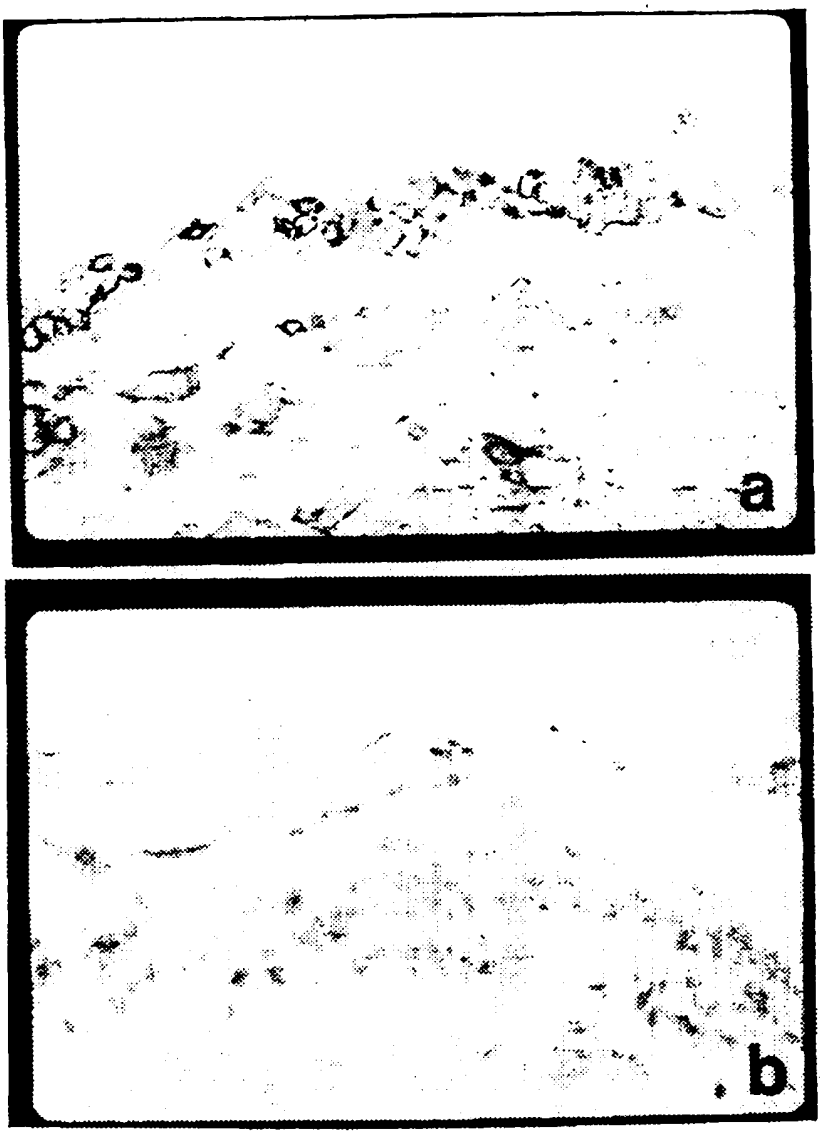

FIGURE 9B

FIGURE 10A

FIGURE 10B

FIGURE 11A

FIGURE 11B

FIGURE 11C

FIGURE 11D

FIGURE 12A

FIGURE 12B

FIGURE 12C

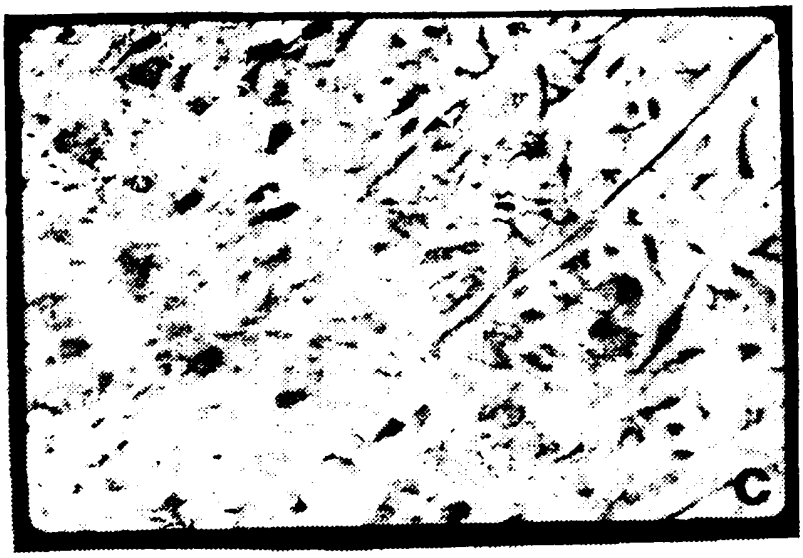

FIGURE 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12925

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 38/02, 38/17, 39/395,

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 141.1, 143.1, 144.1, 153.1, 154.1, 173.1; 514/2, 8, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, CA, EMBASE, MEDLINE, WPI
search terms: cd40, cd40L, cd40 ligand, smooth muscle, bladder, gut, intestine, bowel, gastrointestinal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93/09812 A1 (THE TRUSTEES OF COLUMBIA UNVERSITY IN THE CITY OF NEW YORK) 27 May 1993, see entire document.	1-70
Y	Ann. Rev. Immunol., Volume 12, issued 1994, Banchereau et al., "The CD40 Antigen and Its Ligand", pages 881-922, see entire document, including page 891-892.	1-70
Y	J. Exp. Med., Volume 182, issued December 1995, Yellin et al., "Functional Interactions of T Cells with Endothelial Cells: The Role of CD40L-CD40-mediated Signals", pages 1857-1864, see entire document, including the Discussion.	1-70

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
22 OCTOBER 1997

Date of mailing of the international search report
14 NOV 1997

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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12925

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 133.1, 141.1, 143.1, 144.1, 153.1, 154.1, 173.1; 514/2, 8, 12